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REEVALUATING ESSENTIAL FATTY ACID NUTRITION IN FLORIDA POMPANO, *Trachinotus carolinus*, AND NILE TILAPIA, *Oreochromis niloticus*

By

Christopher J. Jackson

B.S., Southern Illinois University, 2014

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree

Department of Zoology
In the Graduate School
Southern Illinois University Carbondale
May 2017

THESIS APPROVAL

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Approved by:

Dr. Jesse T. Trushenski, Chair

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Graduate School
Southern Illinois University Carbondale
March 31, 2017

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Christopher J. Jackson, for the Master of Science degree in Zoology, presented on March 31, 2017, at Southern Illinois University Carbondale.

TITLE: REEVALUATING ESSENTIAL FATTY ACID NUTRITION IN FLORIDA POMPANO, *Trachinotus carolinus*, AND NILE TILAPIA, *Oreochromis niloticus*

MAJOR PROFESSOR: Dr. Jesse T. Trushenski

Aquaculture is currently the fastest growing sector of protein production, and is expected to overtake the harvest of wild fisheries. Limitations in nutrition, specifically fatty acid nutrition, are preventing even more dramatic growth of many species of commercial importance. Currently, much of the research involving fatty acids examines requirements as being correlated to thermal guilds (warm vs. cool water) or salinity tolerance (marine vs. freshwater). However, recent studies have revealed the potential for trophic level to be as much, if not more, influential in determining fatty acid requirements of a species. As such, two feeding trials were conducted to determine the requirements of two species of different trophic levels (*Oreochromis niloticus* and *Trachinotus carolinus*) based on C_{18} PUFA vs. LC-PUFA. Nile Tilapia, *O. niloticus*, exhibited similar growth regardless of the inclusion of C_{18} PUFA or LC-PUFA, however, tissue fatty acid profiles were influenced per the diet provided. As such, it was concluded that Nile Tilapia exhibit the capacity to effectively synthesize LC-PUFA from C_{18} PUFA as is seen in many species that occupy low trophic levels. Florida Pompano, *T. carolinus*, did not exhibit any significant differences in growth regardless of the diet provided, but numerical differences indicated benefits towards inclusion of dietary LC-PUFAs. Similar to *O. niloticus*, tissue fatty acid profiles were significantly affected by dietary treatment. Based on numerical differences in growth performance and significant differences in tissue fatty acids, it was concluded that Florida Pompano show a typical carnivorous requirement for LC-PUFA.

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LIST OF ABREVIATIONS AND ACRONYMS

ALA – alpha Linolenic acid; 18:3n-3

ARA - Arachidonic acid; 20:4n-6

C_{18} PUFA – 18-carbon or medium-chain polyunsaturated fatty acid; 18 carbon atoms, ≥2 double bonds

CFAAS – Center for Fisheries, Aquaculture and Aquatic Sciences; Carbondale, Illinois

DHA - Docosohexaenoic acid; 22:6n-3

EFA – Essential Fatty Acids

EFAD – Essential Fatty Acid Deficiency

EPA - Eicosapentaenoic acid; 20:5n-3

Fad – Fatty acid desaturase; enzyme responsible for the addition of a single double bond

FAME – Fatty acid methyl ester

FAO – Food and Agriculture Organization of the United Nations

FCR – Feed conversion ratio; feed (dry matter)/weight gain

HSI – Hepatosomatic index

LA - Linoleic Acid; 18:2n-6

LC-PUFA - Long chain polyunsaturated fatty acid; ≥20 carbons, ≥3 double bonds

MUFA – Monounsaturated fatty acid; 1 double bond

NRC – National Research Council

PUFA – polyunsaturated fatty acid; ≥2 double bonds

SFA – Saturated fatty acid; no double bonds

SGR – Specific growth rate

USD – United States dollar

VSI – Viscerosomatic index

CHAPTER 1

INTRODUCTION

Seafood production is currently 167.2 million metric tons annually and is likely to increase even further as the human population is expected to grow by an additional 2 billion people by the year 2050 (FAO 2014). Rising demand for seafood and the increased industrialization of fishing has created enormous harvest pressure, resulting in nearly 90% of all fisheries being classified as fully fished or over fished (FAO 2016). Aquaculture, the culture of fish and other aquatic organisms, has grown dramatically since the 1990s to close the 'seafood gap', i.e., the difference between growing seafood demand and relatively static capture fisheries landings.

Aquaculture production has grown at an average of 8.5% per year over the period of 1950 to 2006 (Tacon and Metian 2008), making it the fastest growing sector of meat production in the world. Due primarily to growth in the aquaculture sector, total annual seafood production (including capture fisheries and aquaculture) has nearly tripled, from approximately 40.8 million metric tons in 1970 to 128 million metric tons in 2010 (Tacon and Metian 2013). Although capture fisheries landings have been relatively static since the 1990s, substantial increases in seafood supply have come from aquaculture, which has increased from 1.47 to 38.5 million metric tons of edible seafood from 1970 to 2010 (Tacon and Metian 2013). In 2014, aquaculture production topped 73 million metric tons (FAO 2016), accounting for 50% of the total global seafood supply, an increase from nearly 20% in 1990 (FAO 2014). This growth in the aquaculture sector is important to maintain as capture fisheries remain relatively static and the human population continues to grow. Due to the human population's anticipated increase in size, aquaculture is expected to continue this trend of increased production and reach 60% of total food fish production by 2030 (FAO 2014).

Although continued growth of the aquaculture sector is necessary, it will be constrained by the availability of inputs, including cost effective ingredients for aquafeeds. Historically, fish meal and fish oil, derived from marine reduction fisheries, were primary sources of protein and lipid in aquafeeds. Fish meal is a result of rendering fish tissue (both whole carcass and offal) down into a useable protein-dense powder. This powder is then processed further to remove much of the oil contained in the fish meal,

creating another product, fish oil. However, like many other capture fisheries, reduction fisheries are considered stable, but unlikely to yield substantially greater landings in the future. Simultaneously, demand for reduction fishery products has increased with the demand for industrially compounded feeds as a result of aquaculture moving away from non-fed, pond, based systems to more intensive culture systems dependent on external nutrients (Tacon et al. 2011; FAO 2012). With this increased demand for complete feeds, the aquaculture industry's consumption of available fish meal and fish oil supplies has increased. Historically, fish oil was used as a primary lipid source because of its acceptance by aquatic species, high energy and long chain polyunsaturated fatty acid (LC-PUFA) content, and competitive pricing (Turchini et al. 2009; Tocher 2015). Due to increasing demand, the cost of these products has been driven ever higher (FAO 2008; Tacon and Metian 2008), with fish oil surpassing $2,000 USD per metric ton in 2013 (FAO 2014). Due to static capture fisheries landings, including reduction fisheries, it is anticipated that the cost of fish oil will continue to rise (FAO 2008, 2014; Tacon and Metian 2008). In 2008, the aquaculture sector consumed 87% of the total fish oil produced for the global market, much of which was destined for the production of high value species (Tacon and Metian 2009). Despite decreased fish oil inclusion rates in aquafeeds, growth in aquafeed manufacturing is expected to drive fish oil demand up by an additional 23 million metric tons (Tacon and Metian 2008; FAO 2012).

Fish oil is an important source of LC-PUFAs, for which all fishes have a physiological demand. As in humans and other vertebrates, LC-PUFAs are needed for reproductive, neurological, vascular, visual and other functions in fish (Glencross 2009). Some fish exhibit a dietary requirement for these nutrients (i.e., they must consume LC-PUFAs directly), whereas others can meet physiological demand through *de novo* synthesis from ingested 18 carbon polyunsaturated fatty acid (C_{18} PUFA) precursors. LC-PUFAs are a product of metabolic pathways that transform C_{18} PUFA, namely linoleic acid (LA) and alpha linolenic acid (ALA), via the actions of a series of desaturase and elongase enzymes to increase the number of carbons and double bonds in the molecule (Sprecher et al. 1995). The production of both n-3 and n-6 LC-PUFAs is dependent on the same series of enzymes, resulting in inadequate synthesis when substrates are limiting or the enzymes are inactive or not present in meaningful amounts (Figure 1.1). A lack of enzymes is thought to be caused by a species' evolutionary adaptation to a diet rich in LC-PUFAs (i.e. carnivorous diet), causing a decreased expression of the necessary elongation and desaturation

enzymes (Carmona-Antoñanzas et al. 2013; Tocher 2015). Dietary composition is used in determining the trophic status of a species, their trophic level. Primary producers being the base of all food webs are given a trophic level of 1, primary consumers (herbivores) are given a trophic level of 2, and secondary consumers (carnivores) have a trophic level of 3. Carnivores that prey on other carnivores and apex predators are assigned to trophic levels of 4 and 5, respectively.

If a species were to have evolved utilizing a more herbivorous or omnivorous diet largely deficient in LC-PUFAs, presumably organisms would need to maintain the ability to produce LC-PUFAs from abundant dietary C_{18} PUFAs (Tocher 2010, 2015; Monroig et al. 2011). Some of the most globally important aquaculture species are thought to have no dietary requreiment for LC-PUFAs; such as carp which account for 72% of global freshwater aquaculture production (FAO 2012) and the most widely produced species globally, tilapia (FAO 2012). However, there is evidence that total dietary demand for fatty acids can be reduced through the inclusion of intact LC-PUFAs in the diet (Tocher 2010). In contrast to herbivores and omnivores, most carnivorous fish evolved consuming diets already rich in preformed LC-PUFAs. Consequently, there would be little selective advantage in possessing the ability to synthesize these nutrients *de novo*. Having lost, or never acquired, the ability to produce necessary enzymes in meaningful amounts, Red Seabream, *Pagrus major* (Yone and Fuji 1975; Wantanabe 1982; Takeuchi et al. 1992), Yellowtail, *Seriola* sp. (Furukawa et al. 1966; Tsukuhara et al. 1967; Deshimaru et al. 1982) and many other carnivorous species are unable to synthesize LC-PUFAs from C_{18} PUFAs and therefore require intact LC-PUFAs in their diet (NRC 2011).

The capacity of a fish species to synthesize LC-PUFAs from C_{18} PUFA precursors largely determines whether dietary fish oil sparing (i.e. the replacement of some or all of the fish oil with alternative, less expensive, lipid sources) can be effectively achieved. Whereas plant oils (canola, coconut, corn, olive, palm, peanut, soybean, etc.) do not offer LC-PUFAs, they typically contain C_{18} PUFAs that could be converted into biologically active LC-PUFAs (Figure 1.1) (NRC 2011). In addition to the wide variety of plant-based lipids, many of the terrestrial animals produced for human consumption have significant amounts of trimmings and waste that can be rendered down to usable sources of lipid; these lipid sources include beef tallow, poultry fat, and pork lard (Turchini et al. 2009). Terrestrial animal

fats are mainly comprised of mono-unsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs), both of which are known to be preferentially catabolized for energy compared to polyunsaturated fatty acids (NRC 2011). The utilization of these alternative lipids is a well-established method of reducing the inclusion rates of fish oil (Turchini et al. 2009); however, the results of fish oil sparing or complete replacement vary among lipid sources and fish species. For example, Gilthead Seabream (*Sparus aurata*) and European Seabass (*Dicentrarchus labrax*) fed vegetable oil based diets showed no significant differences in production performance (Izquierdo et al. 2003), however, Australian Snapper (*Pagrus auratus*) exhibited significantly reduced growth when fed diets of crude or refined canola oil (Glencross 2003). Regarding the species of interest in the present work, see *Chapter 2* for information regarding the effects of fish oil sparing in Nile Tilapia, *Oreochromis niloticus*, and *Chapter 3* for information regarding Florida Pompano, *Trachinotus carolinus.* Although fish oil sparing has significant impacts on the cost of aquafeed production, there are complications associated with it. Due to the absence of LC-PUFAs, fish oil sparing can result in fatty acid deficiencies in species unable to synthesize LC-PUFAs *de novo*. Consequently, species lacking the necessary enzymes are particularly prone to reductions in growth, feed efficiency, and feed intake as well as tissue fatty acid profiles that are distorted when compared to a fish oil fed counterpart (Trushenski et al. 2009, 2012, 2013; Woitel et al. 2014a; Bowzer et al. 2016; Emery et al. 2016). However, with the inclusion of LC-PUFAs directly into alternative lipid diets or through the use of fish oil-rich finishing feeds at the end of production cycles, it is possible to restore much of the production performance and fatty acid profiles to associated with fish oil based diets (Trushenski 2012; Bowzer et al. 2016; Emery et al. 2016).

Knowledge of fatty acid requirements is central to successful sparing or replacement of fish oil in aquafeeds, but information regarding the demands for lipids and fatty acids in many commercially important species is lacking. Much of the ‘common knowledge’ regarding fatty acid requirements of fish is based on the notion that temperature and salinity guilds are the driving causes for different fatty acid essentiality, i.e., whether C_{18} PUFAs or LC-PUFAs are required in the diet. According to these conventions, coldwater and marine fish require LC-PUFAs, whereas warmwater and freshwater fish do not; however, quantitative investigations have proven that these ‘rules of thumb’ are regularly inaccurate. Other studies report “total n-3”, “total n-6”, or merely lipid inclusion rates opposed to individual fatty acids.

Fatty acid groupings do offer insight on the requirements of species, however, they do not offer sufficient information to develop diets that maximize the production of their respective species. It is very likely that due to evolutionary forces, omnivorous species can consume a diet containing C_{18} PUFAs resulting in growth comparable to diets that contain a full suite of LC-PUFAs. On the other hand, it is unlikely that retaining or acquiring the ability to synthesize LC-PUFAs would be of considerable selective advantage among species consuming food items rich in these nutrients. Fish nutritionists specializing in lipid demand and fatty acid requirements increasingly recognize the likely importance of trophic level as a predictor of whether a species exhibits a dietary requirement for LC-PUFAs. However, much of the literature and associated recommendations continue to focus on the aforementioned temperature/salinity-based conventions or 'requirements' for fatty acid groupings. The purpose of this thesis was to address the fatty acid requirement debate in commercially relevant aquaculture species, assess the essentiality of C_{18} PUFA or LC-PUFA in feeds for taxa representing freshwater/marine and omnivore/carnivore dichotomies, and facilitate better use of trophic level as predictor of fatty acid requirements of fish.

CHAPTER 2

REEVALUATING ESSENTIAL FATTY ACID REQUIREMENTS OF NILE TILAPIA

Nile Tilapia inhabit tropical, shallow waterways and are highly adaptable to a wide range of water conditions (e.g. temperature, dissolved oxygen, salinity, nitrogenous compounds) (Popma and Masser 1999). In addition to their tolerance of varied environmental conditions, Nile Tilapia are well-suited to aquaculture because they are fecund and reproduce readily in intensive and extensive rearing systems, accept a wide variety of feed ingredients, exhibit rapid growth rates, and yield a mildly flavored fillet that is desirable to consumers. As a result, tilapias are the most widely cultured aquatic species worldwide (FAO 2014), with production of Nile Tilapia exceeding 3.6 million metric tons in 2013 (FAO 2017). The production of this fish occurs on multiple continents, but many of the producing countries are in Southeast Asia. Feed constitutes 45-80% of tilapia farm operational costs, and there is great desire to reduce these costs to increase profitability (Ng and Chong 2004). Protein sources are typically the most costly components of aquafeeds, but identification of suitable, low-cost sources of lipids are also needed in order to satisfy dietary demands for energy and essential fatty acids.

The reported essential fatty acid requirements of tilapia are relatively easy to satisfy, and thus various lipid sources, including fish oil, plant oils, and terrestrial animal fats, have been used successfully in tilapia diets. Utilization of alternative lipid sources has resulted similar or better growth performance than fish oil when provided to tilapia (Ng et al. 2001; Mulligan and Trushenski 2013). Red hybrid tilapia, *Oreochromis* sp., fed diets containing cod liver oil grew significantly less efficiently than those fed diets containing crude palm oil (Ng et al. 2001). Blue Tilapia, *Oreochromis aureus*, exhibits a similar capacity for utilizing alternative lipid sources: fish fed diets containing menhaden oil, catfish oil, and soybean oil all exhibited similar performance, however, beef tallow resulted in reduced growth (Stickney and McGeachin 1983). The ability to utilize different lipid sources is due to the ability of tilapias to synthesize LC-PUFAs from C_{18} PUFAs (Tocher et al. 2002). Nile Tilapia have a reported dietary requirement for LA (Teshima et al. 1982; NRC 2011), but there are indications that direct provision of ARA may be a more efficient means of satisfying physiological demand for this n-6 LC-PUFA (Takeuchi et al. 1983). No quantitative requirement for n-3 fatty acids has been reported (NRC 2011), but beneficial effects of having both n-3

and n-6 fatty acids in the diet of hybrid tilapia have been reported (Chou and Shiau 1999) and there is little reason to think that tilapias are unique among vertebrates and that n-3 fatty acids are completely dispensable for these taxa. Taken together, these reports suggest that the essential fatty acid requirements of tilapia are perhaps not as well-understood as one might expect, given the size of the commercial industry. Although the performance of Nile Tilapia is maintained regardless of the source of lipid being used, the fillet tissue fatty acid profile does mimic the profile of the oil being fed (Chou and Shiau 1999; Ng et al. 2001; Ng and Chong 2004).

In order to facilitate fish oil sparing and the flexibility to incorporate a variety of different lipid sources in aquafeeds, fish nutritionists and feed manufacturers must know which fatty acids are essential and what dietary levels are adequate to satisfy requirements of the intended fish. As such, a more accurate understanding of essential fatty acid (EFA) requirements of fish is instrumental to the continued growth of global aquaculture. Given the wide variety of species cultured throughout the world, it is infeasible to conduct quantitative essential fatty acid requirements in all species. 'Rules of thumb' or information to help nutritionists predict the dietary requirements of untested species are needed. In the past, nutritionists attempted to use species' preferred water temperatures (warm-, cool-, or coldwater) or salinities (freshwater or saltwater) to predict essential fatty acid requirements (e.g., it has been suggested that coldwater/saltwater fish require LC-PUFAs, whereas warmwater/freshwater taxa do not), but these guidelines have proven inaccurate in many cases. More recently, it has been suggested that trophic level may be a better predictor of a fish's ability to synthesize LC-PUFAs *de novo* or whether they must consume them directly. In order to assess this hypothesis I used Nile Tilapia, a freshwater herbivore (trophic level 2.0) (Froese and Pauly 2016), to examine fatty acid requirements on the basis of C_{18} PUFA or LC-PUFA dietary inclusion.

MATERIALS AND METHODS

PREPARATION AND ANALYSIS OF DIETS

Seven dietary treatments were formulated based on a previously verified diet (Trushenski et al. 2009; Mulligan and Trushenski 2013), including a positive control, negative control, and five experimental

treatments amended with C_{18} PUFA or LC-PUFA ethyl esters (Table 2.1). The positive control ("Fish Oil Control") was formulated to include menhaden fish oil (Virginia Prime Gold™, Omega Protein Corporation, Houston, Texas) as the single lipid source. The negative control ("EFA Free Control") was formulated using hydrogenated soybean oil (Dritexs Shortening Flakes, Stratas Foods, LLC, Memphis, Tennessee) in place of menhaden oil. Five experimental diets were prepared by amending the EFA Free Control formulation with ALA ("ALA"); ALA and LA ("C_{18} PUFA"); docosohexaenoic acid ("DHA"); DHA and arachidonic acid (ARA) ("DHA + ARA"); or ARA, eicosapentaenoic acid (EPA), and DHA ("LC-PUFA"). All diets were formulated using solvent extracted fish meal to avoid contamination from residual fish oil. However, due to residual lipid content present in other ingredients, LA was present in all diets, including the EFA Free Control and other experimental diets not supplemented with LA ethyl esters (Table 2.2, 2.3).

All diets were prepared at the Center for Fisheries, Aquaculture and Aquatic Sciences (CFAAS) using established internal practices. Briefly, ingredients were combined using a cutter mixer (Model CM450, Hobart Corporation, Troy, Ohio) and then pelleted using a commercial feed grinder (1.5 h.p. grinder, Cabela's, Sydney, Nebraska). After pelleting, the feeds were dried at 100°C in a commercial dehydrator (Harvest Saver R-5A, Commercial Dehydrator Systems, Eugene, Oregon). All diets were stored frozen (-20°C) throughout the duration of the trials, prior to commencement of the trial triplicate samples of each diet were collected and lyophilized (Freezone6, Labconco, Kansas City, Missouri) for the determination of moisture content. The samples were then pulverized for further analysis of ash, protein, and lipid content. Ash content of all diets was determined using a muffle furnace set to 650°C for 4 hours and allowed to cool overnight. Protein content was determined using a combustion method of nitrogen determination (LECO® FP-528, LECO Corporation, St. Joseph, Michigan). Lipid content was determined gravimetrically via chloroform/methanol extraction (Folch et al. 1957), followed by acid transmethylation for fatty acid determination (Christie 1982). Quantification of FAMEs was achieved via gas chromatograph equipped with a flame ionization detector (GC-FID) (GC-17A, Shimadzu Kyoto, Japan) fitted with a fused silica capillary column (30m x 0.25mm interior diameter) (Omegawax 250, Supelco, Bellefonte, Pennsylvania). Fatty acid methyl esters were identified by comparison to external standards (Supelco 37

FAME component, PUFA-1, PUFA-3, DHA, ARA, Supelco, Bellefonte, Pennsylvania; 20:3n-9, 22:5n-6, Cayman Chemical Company, Ann Arbor, Michigan).

EXPERIMENTAL DESIGN

Juvenile phenotypically male (sex-reversed) Nile Tilapia were sourced from Americulture, Inc. (Animas, New Mexico) at approximately 0.5 g/fish. A recirculating system consisting of 28, 150 L fiberglass aquaria and associated mechanical and biological filtration at the CFAAS was stocked with 10 randomly selected fish (25.7 ± 0.2 g/fish; mean ± SE). Prior to commencement of the trial all fish were raised on a commercially available diet. All diets were randomly assigned to quadruplicate tanks (*N=4*) for each trial, with each tank being fed to satiation twice daily for 7 weeks. Temperature and dissolved oxygen were monitored daily using a Hach HQ40d meter (Hach; Loveland, Colorado), monitoring of ammonia, nitrite, nitrate and alkalinity was conducted via spectrophotometric analysis (Hach; Loveland, Colorado). Water quality parameters were maintained within ranges suitable for Nile Tilapia rearing: temperature = 27.7 ± 0.7℃; dissolved oxygen = 5.5 ± 0.6 mg/L; total ammonia = 0.03 ± 0.02 mg/L; nitrite = 0.05 ± 0.03 mg/L; nitrate = 39.0 ± 9.9 mg/L; alkalinity = 110.3 ± 43.8 mg/L (mean ± SD).

SAMPLE COLLECTION AND ANALYSIS

At the conclusion of the trial, all fish from each tank were euthanized using an overdose of tricaine methanesulfonate (MS-222; >200 mg/L until cessation of opercular movements for ~ 5 min) (Figure 2.1). Individual fish were weighed and measured (total length), and 5 fish were randomly selected for tissue collection and stored frozen (-20°C) until dissection. Production performance metrics were calculated as follows:

$$\text{Weight Gain (\%)} = 100 \times \frac{(\text{average final individual weight} - \text{average initial individual weight})}{\text{average initial individual weight}}$$

$$\text{Feed Intake (\% body weight/d)} =$$

$$100 \times \frac{\text{average individual dry matter feed intake}}{(\text{average initial individual weight} \times \text{average final individual weight})^{0.5} \Big/ \text{days of feeding}}$$

$$\text{Specific Growth Rate (SGR, \% body weight/d)} = 100 \times \frac{\log_e \text{average final weight} - \log_e \text{average initial weight}}{\text{days of feeding}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{average individual dry matter feed intake}}{\text{average individual weight gain}}$$

$$\text{HSI} = 100 \times (\text{liver weight} / \text{whole body weight})$$

$$\text{VSI} = 100 \times (\text{viscera weight} / \text{whole body weight})$$

Samples of liver, white muscle, intraperitoneal fat (IP fat), brain, and eye tissues were collected from each fish dissected and stored frozen (-80°C) until fatty acid analysis. Muscle samples were lyophilized and pulverized in the same manner described above for diet samples, and then subjected to chloroform/methanol lipid extraction (Folch 1957). All other tissues were homogenized directly in solvent (PowerGen 1000 homogenizer; Fisher Scientific, Pittsburgh, PA) without lyophilization prior to lipid extraction. Resulting total lipid fractions were then subjected to transmethylation and FAME quantification as described above.

Essential fatty acid deficiency indicator ratios were calculated for all tissues, including 20:3n-9 : 20:4n-6 and 22:5n-6 : 22:6n-3. These ratios have been implicated in assessing the relative deficiencies that can arise when EFA are not provided in appropriate amounts (Galli et al. 1974; Siguel et al. 1987). Long-chain PUFA are formed using the same series of enzymes, resulting in the formation of fatty acids that are of lower biological value. Identification of an ARA deficiency is accomplished by comparing the values of 20:4n-6 and 20:3n-9, both of which utilize Δ5 desaturase. The formation of 22:5n-6 and 22:6n-3 utilizes the Δ6 desaturase, with an increased ratio value indicating a deficient diet. By comparing the relative amounts of these fatty acids, deficient diets could be identified prior to physical symptoms arising.

Coefficient of Distance (Djh) values (Turchini et al. 2006), comparing differences in tissue fatty acid profiles between all treatments and the Fish Oil Control diet, were calculated as follows:

$$Djh = \left[\sum_{i=1}^{n} (Pij - Pih)^2 \right]^{1/2}$$

Where Pij represents the percent of fatty acid “i” present in the positive control treatment and Pih represents the percent of fatty acid “i” present in an experimental treatment. Only major fatty acids (>1% of total quantified FAME; no fatty acid groupings e.g. SFAs, MUFAs) were included in the calculation of Djh. Based on these calculations, a smaller Djh value represents tissue profile similarity between treatments, and a value of zero indicates identical tissue profiles.

STATISTICAL ANALYSIS

One-way ANOVA procedures were used to analyze all production performance metrics, and fatty acid data (PROC GLIMMIX; SAS version 9.4, SAS Institute, Cary, North Carolina) with tanks serving as experimental units (N=4). When omnibus tests indicated significant treatment effects, post-hoc Tukey’s HSD tests were used for pairwise comparisons of treatment means. In all cases, effects and differences were considered significantly different at critical values less than or equal to 0.05. Coefficient of distance values were not subjected to formal statistical analysis because of insufficient numbers of replicates (Djh was calculated from fatty acid means, resulting in a single value for each tissue within a treatment).

RESULTS

Although survival did not vary among treatment groups (98-100%), dietary lipid source and fatty acid composition significantly affected other aspects of production performance (Table 2.4). Weight gain, FCR and SGR were significantly greater among fish fed the Fish Oil Control feed in comparison with those fed the EFA Free Control feed. The addition of fatty acid ethyl esters generally improved performance in most cases: growth was sufficiently enhanced to achieve equivalence with the Fish Oil Control treatment group in all experimental groups except DHA and ARA + DHA, but FCR was only significantly improved among fish fed the C_{18} PUFA diet. Feed intake, hepatosomatic index (HSI), and viscerosomatic index (VSI) did not vary among treatments.

Fatty acid composition of tissues varied significantly among treatments, generally reflecting dietary fatty acid profile (see Table 2.2 for dietary fatty acid composition, and Tables 2.5 – 2.9 for tissue fatty acid composition). Levels of SFAs were elevated in all experimental diets relative to the Fish Oil

control, however, these fatty acids were not proportionately incorporated into all tissues. Other fatty acids including ALA, LA, ARA, DHA, and EPA were more consistently reflected in tissues when incorporated into the dietary treatments. Increased levels of ARA were observed in all experimental treatments across multiple tissues (i.e. fillet, liver, brain, and eye) (Tables 2.5, 2.6, 2.7, 2.8, 2.9). Brain tissue with depressed levels of DHA was noted in fish receiving the C_{18} PUFA diet compared to all other diets (Table 2.8). Intraperitoneal fat (Table 2.7) exhibited similar patterns as seen in fillet tissue, except for a significant increase in ARA. Brain (Table 2.8) tissue exhibited similar trends as other tissues in regards to reflecting the dietary consumption, but with much less distortion than all other tissues (Table 2.10).

In all tissues, with the exception of IP fat, the highest values for 20:3n-9 : 20:4n-6 were observed in the DHA treatment, followed by treatments not provided with n-6 fatty acids (i.e. ALA, C_{18} PUFA, and EFA-Free Control). However, in the IP fat samples, the highest values of this ratio were present in the treatments containing LC-PUFAs (i.e. DHA, ARA+DHA, and LC-PUFA) as well as the EFA-Free Control. The ratio between 22:5n-6 and 22:6n-3 in treatment groups provided with diets having a ratio of n-3 to n-6 greater than 1.2 was consistently lower across all tissues compared to treatment groups provided diets with a greater proportion of n-6 fatty acids.

DISCUSSION

Provision of intact LC-PUFA to juvenile Nile Tilapia resulted in no significant effect on growth compared to diets containing C_{18} PUFA. The ability to grow and develop properly when provided diets lacking LC-PUFA has been reported previously in trials with tilapia (Teshima 1982; Chou and Shiau 1999; Trushenski et al. 2009). Based on the numerical differences in my trial, there is evidence supporting the current fatty acid recommendations (NRC 2011) for Nile Tilapia requirement of LA. Although the EFA-Free control contained more LA than the reported requirement (NRC 2011), both the ALA and C_{18} PUFA diets performed better. The growth benefit associated with the combination of both n-3 and n-6 fatty acids has been implied previously in hybrid tilapia (*O. niloticus* x. *O. aureus*) (Chou and Shiau 1999).

Reflection of dietary fatty acid intake in tissues has been reported in numerous species (Craig and Gatlin 1995; Chou and Shiau 1999; Trushenski et al. 2013; Bowzer et al. 2016). The present study indicates that Nile Tilapia might benefit from both dietary n-3 and n-6 fatty acids as first suggested by Chou and Shiau (1999). Tissues most affected by the alteration of dietary fatty acids were those reported by others to be the most compositionally plastic (i.e. fillet, liver, intraperitoneal fat, and, to a lesser extent, eye tissue); brain tissue profiles were the least distorted as a result of dietary manipulation. However, comparing ALA and C_{18} PUFA treatments indicated a lower distortion of all tissues in fish fed a diet rich in ALA (Table 2.10). This is further evidenced by the DHA and LC-PUFA diets having the least tissue distortion among all diets.

Among fish fed the EFA Free Control feed, in which LA was the only PUFA present in appreciable amounts, there was a significantly higher level of ARA present in muscle tissue; however, this level dropped in the presence of ALA in the diet (ALA and C_{18} PUFA), as well as in diets containing DHA. The provision of intact n-3 LC-PUFA (namely EPA and DHA) has been implicated in the down regulation of many enzymes responsible for LC-PUFA biosynthesis (Clarke 2001). Thus, it is possible that increased availability of DHA in excess of physiological demand among fish fed the DHA-supplemented feeds induced a down-regulation of Δ6 Fad, indirectly resulting in less ARA synthesis and deposition (Chou et al. 1999). In the case of the ALA and C_{18} PUFA diets, the presence of a competing substrate, i.e., ALA, may have effectively reduced ARA synthesis from LA.

When provided with n-3 fatty acids (ALA and DHA), tissue 20:3n-9/20:4n-6 ratios declined in Nile Tilapia. Synthesis of 20:3n-9 typically occurs in the absence of biologically active 20-carbon PUFA, but the organism is unable to synthesize the needed fatty acids. The use of the Δ5 desaturase enzyme complex is required for the proper desaturation of both 20:4n-3 and 20:3n-6 to 20:5n-3 (EPA) and 20:4n-6 (ARA), respectfully (Figure 1.1). Once the physiological demand for EPA, or more specifically DHA, was met in treatments amended with either ALA or DHA, the conversion of all precursors was dramatically reduced. Thus, an increase in 20:3n-9/20:4n-6 ratio indicates a developing or ongoing essential fatty acid deficiency (namely LA). The results of this study appear to indicate that in a diet containing mainly LA (EFA-Free control; Table 2.3) Nile Tilapia produce significantly more 20:3n-9 than when provided with

ALA and LA simultaneously. This finding, coupled with the growth performance results I observed suggests that Nile Tilapia do indeed have a dietary requirement for n-3 PUFAs, but that this requirement can be met through provision of ALA and that dietary provision of n-3 LC-PUFA is not explicitly necessary. These results are consistent with the understanding that fatty acids are synthesized in an n-3 > n-6 > n-9 fashion (Tocher 2010). Although significant differences in both fatty acid deficiency indicator ratios (20:3n-9 : 20:4n-6, and 22:5n-6 : 22:6n-3) in all tissues were present, no fish showed any gross indications of fatty acid deficiency. Fish fed diets amended with high levels of n-3 fatty acids (i.e. ALA, DHA, and LC-PUFA) consistently experienced higher ratios of 20:3n-9 to 20:4n-6, as well as lower levels of 22:5n-6 to 22:6n-3. Alternatively, treatments with high levels of n-6 fatty acids (i.e. EFA-FREE, C_{18} PUFA, and ARA) exhibited low levels of 20:3n-9/20:4n-6 and high levels of 22:5n-6/22:6n-3. Typically, a 20:3n-9/20:4n-6 ratio of >0.2 is considered indicative of a fatty acid deficiency in mammals, however, it is not until the ratio surpasses 0.4 that clinical signs are exhibited (Holman 1971a, 1971b, 1978). Using a 20:3n-9/20:4n-6 ratio value of >0.2 no experimental treatments experienced acute fatty acid deficiency in fillet tissues, however, intraperitoneal fat from all diets containing LC-PUFA (i.e. DHA, ARA+DHA, and LC-PUFA) all exhibited ratio values of >0.9. As IP fat is understood to act as storage for excess lipid, and subsequently fatty acids, this tissue might not be the most informative when determining essential fatty acid deficiency (EFAD). Although no value for determining a fatty acid deficiency with a 22:5n-5/22:6n-3 ratio, it has been suggested that higher levels indicate declining health (Galli et al. 1974). Based on my results there are clear indications that in treatments provided with n-3 fatty acids the values of 22:5n-6/22:6n-3 are much lower than in treatments provided with n-6 fatty acids.

As a species with a low trophic classification, Nile Tilapia (trophic level = 2.0 (Froese and Pauly 2016) are one of the most widely produced species globally (FAO 2014). Low trophic level species, including Nile Tilapia, Common Carp (trophic level 3.0) (Froese and Pauly 2016) and catfish (including *Pangasius,* trophic levels = 3.1 - 3.4) (Froese and Pauly 2016) are expected to account for approximately 60% of global aquaculture production by 2025 (FAO 2016). Common carp, a cool, freshwater species (trophic level = 3.0) (Froese and Pauly 2016) is reported as having a dietary requirement for both ALA and LA (Takeuchi and Wantanabe 1977). Grass carp (trophic level = 2.0) (Froese and Pauly 2016) are a warm, freshwater species reported to have a similar requirement of both ALA and LA (Takeuchi et al.

1991). Ayu (*Plecoglossus altivelus*) is a coolwater, catadromous species (trophic level = 3.0) (Froese and Pauly 2016) with reported fatty acid requirements of ALA (Kanazawa et al. 1982). Combined with the results of my trial, there appears to be a trend indicating that fish species adapted to low trophic levels, regardless of thermal or salinity preference, can utilize dietary C_{18} PUFA to produce and satisfy physiological demand for derivative LC-PUFAs. This trend can be a useful start when determining a potential aquaculture species' fatty acid requirements.

Further research is needed to determine if the numerical differences shown in this trial were indeed non-significant, or if given a longer period of time significant differences would develop. Although sufficient statistical power was present, a longer trial length would allow for additional growth and further separation of treatment effects. As hypothesized, Nile Tilapia appear to have a dietary requirement for C_{18} PUFA likely due to their low trophic level. Additional trials are required to determine the specific levels that are required for the optimal growth of juvenile Nile Tilapia, followed by advanced stages of growth and eventually reproduction as these stages will likely have different fatty acid requirements. Additional studies of species with different trophic levels will provide additional information regarding the use of trophic level as an indicator of fatty acid requirements.

CHAPTER 3

EVALUATION OF ESSENTIAL FATTY ACID DEMANDS OF FLORIDA POMPANO

Florida Pompano, *Trachinotus carolinus*, is a tropical, marine carnivorous fish. Florida Pompano are a coastal, shallow water, pelagic species that are commonly found in schools near beaches, as well as in bays and estuaries (Main et al. 2007). Interest in Florida Pompano culture has been driven by the species' tolerance for varied dissolved oxygen and salinity levels, iteroparity and ability to spawn throughout the year, and rapid growth (Main et al. 2007; Riley and Weirich 2010), as well as the popularity of pompano fillets due to their light flavor and flaky texture (Main et al. 2007). High demand coupled with limited supply has led to retail prices of Florida Pompano ranging between $20.00 and $31.11 per kg in 2014 (FAO 2017). Despite the high prices and demand for Florida Pompano, currently there is limited commercial fishing or aquaculture production (Main et al. 2007). Currently, the considerable potential of Florida Pompano culture is constrained by the comparatively limited information available to support optimization of husbandry practices, including nutrition and feeding. Quantitative nutrient requirements are generally lacking and recommendations regarding feed selection and feeding practices are general and largely adapted from information available for other species.

Much of the information regarding Florida Pompano has focused on bulk lipid demand, with very limited information regarding fatty acid requirements. However, the information available suggests that fish oil, or rather the LC-PUFAs fish oil provides, are especially important in Florida Pompano feeds. For example, growth of juvenile Florida Pompano was directly related to dietary inclusion rate of fish oil, with a range of 234 – 826% weight gain when fed 0, 4, 8, or 12% fish oil (Williams et al. 1985). The addition of 8% fish oil resulted in the highest weight gain (826%) as well as the most efficient FCR of 1.86 (Williams et al. 1985). The addition of fish oil, in addition to enhancing growth, resulted in significantly higher survival and the prevention of growth abnormalities including lesions of gills and operculum (Williams et al. 1985), suggesting that essential fatty acid levels were the limiting factor in the other, lower fish oil inclusion diets. These authors cited the presence of LC-PUFAs present in fish oil, specifically EPA and DHA, and attributed the differential performance they observed to the relative abundance of these nutrients in Florida Pompano diets.

Although the work of Williams et al. (1985) is suggestive, it does not definitively prove that LC-PUFAs are required nutrients in Florida Pompano feeds as there is evidence that Pompano are capable of utilizing alternative lipid sources not containing LC-PUFAs. It was recently shown that the replacement of 75% of dietary fish oil can be successful with multiple plant based oils (Rombenso et al. 2016a). In these trials, juvenile fish were successfully raised for 8 weeks on alternative lipids with no significant differences in weight gain and, although significant, only small differences in feed conversion ratios. However, Pompano do show a trend (Rombenso et al. 2016a) similar to other marine species (Trushenski et al. 2011, 2012, 2013; Woitel et al. 2014a, 2014b) of altered fillet fatty acid composition according to the fatty acids provided in the diet. The capacity for fish oil sparing in this species is further supported by the complete replacement of fish oil with beef tallow (Rombenso et al. 2016b). This study also implied greater importance of DHA over EPA in the growth of Pompano, a result that has been identified in several species of carnivorous species (Trushenski et al. 2012; Bowzer et al. 2016, Emery et al. 2016).

The limited information available appears to indicate that Florida Pompano, like many other marine carnivores, exhibit a dietary requirement for LC-PUFAs. All vertebrate animals exhibit physiological demand for ARA, EPA, and DHA, but many are able to meet this physiological demand through *de novo* synthesis of LC-PUFAs from C18 PUFA precursors, i.e., LA and ALA. For these species, including various herbivorous or omnivorous fishes (Benitez and Gorriceta 1985; Radunz-Neto et al. 1996; Xu and Kestemont 2002; Lim et al. 2011; Morais et al. 2012), a diet with adequate levels of LA and ALA is sufficient to support growth and survival. For other species, including many marine carnivores and possibly Florida Pompano, such biosynthetic transformations cannot be completed at a rate sufficient to meet physiological demand for LC-PUFAs. As such, they must be provided with these nutrients in the diet to ensure survival and normal growth and development. Knowing whether C18 PUFAs and/or LC-PUFAs are required in the diet of Florida Pompano is central to proper dietary formulation and least-cost feeding of this species. Accordingly, I evaluated fatty acid requirements of Florida Pompano fed diets containing C_{18} PUFAs or LC-PUFAs.

METHODS

PREPARATION AND ANALYSIS OF DIETS

Seven dietary treatments generally consistent with diets used in previous Florida Pompano research were formulated in the same manner as described in Chapter 2. Preparation of diets was conducted the same as described in Chapter 2, as was determination of proximate and fatty acid composition (Table 3.2).

EXPERIMENTAL DESIGN

Juvenile Florida Pompano were obtained from ProAquatix (Vero Beach, FL) and raised on a commercial diet until of an appropriate size able to consume the experimental diets. A recirculating system consisting of 24 (of which 21 were used), 300 L fiberglass aquaria and associated mechanical and biological filtration at the Virginia Seafood Agricultural Research and Extension Center (VSAREC) was stocked with 8 randomly selected fish (47.4 ± 0.6 g/fish; mean ± SE). All diets were randomly assigned to triplicate tanks (*N*=3), with each tank being fed a fixed ration (4% of body weight) split between two daily feedings for 7 weeks; fish were weighed every two weeks to adjust feeding rates for growth. Temperature and dissolved oxygen was monitored daily using a YSI 550 meter (YSI, Inc.; Yellow Springs, Ohio), monitoring of ammonia, nitrite, nitrate and alkalinity was conducted via spectrophotometric analysis (Hach; Loveland, Colorado). Water quality parameters were maintained within ranges suitable for Florida Pompano (Pfeiffer and Riche 2011) rearing: temperature = 27.3 ± 0.3°C; dissolved oxygen = 6.9 ± 0.4 mg/L; total ammonia = 0.50 ± 0.87 mg/L; nitrite = 0.13 ± 0.12 mg/L; nitrate = 17.26 ± 9.58 mg/L; alkalinity = 148.9 ± 18.4 mg/L; salinity = 22.5 ± 0.9 ppt.

SAMPLE COLLECTION AND ANALYSIS

At the conclusion of the trial, group weights were recorded from each tank, with 5 fish from each tank were euthanized using an overdose of tricaine methanesulfonate (MS-222; >200 mg/L) (Figure 2.1). Euthanized fish were then frozen at -10°C overnight, followed by -80°C for three days, after which they were packed with dry ice and shipped to the CFAAS overnight. Upon arrival at the CFAAS, weights and lengths were recorded and all tissues for analysis were collected. All other methods of tissue collection and analysis were performed in the same manner as Chapter 2, with the exception that only 3 samples from each tank were used in the tissue analysis.

RESULTS

Although no significant differences in production performance (i.e. weight gain, FCR, SGR, and FI) existed between the EFA-Free control and experimental diets, there were significant differences between the Fish-Oil Control and all other treatments (Table 3.4). Significant differences in HSI were observed between the ALA diet and the ARA+DHA and LC-PUFA diets. Although no statistical differences were noted among experimental diets, there were numerical trends indicating benefits when including intact LC-PUFA (specifically ARA and DHA).

Fatty acid composition varied significantly between all tissues, generally reflecting dietary fatty acid profiles (see Table 3.2 for dietary fatty acid composition, and Tables 3.5 – 3.9 for tissue composition). Levels of SFAs were elevated in all experimental diets relative to the Fish Oil Control, however, these fatty acids were only slightly different among fillet tissues. Alternatively, levels of MUFAs were reduced in all experimental diets relative to the Fish Oil Control, but these fatty acids were generally elevated in fillet tissues of experimental treatments. Fillet tissues all exhibited higher levels of LC-PUFA compared to C_{18} PUFA when provided in the diet (i.e. Fish Oil Control, DHA, ARA+DHA, and LC-PUFA diets) (Table 3.5). There were no detectable levels of 20:3n-9 in any liver samples, and very low levels in the other tissues sampled, with many of the dietary treatments showing no detectable levels. Levels of 22:5n-6 were noted in all dietary treatments, with significantly higher levels of 22:5n-6 : 22:6n-3 in fish fed the ARA+DHA diet compared to all other treatment groups.

Dietary treatment affected the fatty acid profile of all tissues, but the degree to which fatty acid profile was affected differed among tissues (Tables 3.5 – 3.9). However, as the LC-PUFA content of the diets increased there was a reduction in the total tissue distortion, as shown by the coefficient of distance values (Table 3.9). The coefficient of distance values were largest among fish fed the negative control (EFA Free Control) as well as ALA and C_{18} PUFA diets. However, there was a dramatic decrease when DHA was added, with additional decreases in distance from the reference treatment when ARA, or ARA and EPA were added to the DHA. The only tissue that showed to have minimal distortion from dietary treatment was the brain, with a maximum distortion of only 4.0 (EFA-Free control) and a minimum distortion of 2.0 (DHA).

DISCUSSION

Reflection of dietary fatty acids is a well-documented phenomenon (Sargent et al. 1989, 2002; Bell 1998) shown to exist in a variety of fish species. In the current study, levels of fatty acids present in tissues were significantly affected by dietary treatments. Most notably, significant increases in LC-PUFA content were present when fish were provided with dietary ARA, DHA, and EPA. Equivalent increases in tissue levels of LC-PUFAs were not associated with increasing dietary provision of LA and/or ALA, suggesting limited elongation/desaturation activity. Rombenso et al. (2016a) reported similar fatty acid results in Florida Pompano, with reduced levels of LC-PUFA despite the presence of the respective 18 carbon precursors. European Seabass exhibited reduced levels of LC-PUFA when fed diets containing vegetable oil as a replacement for fish oil (Mourente et al. 2006). Fatty acid content of Cobia fillet tissue has been shown to be negatively affected when a diet deficient in LC-PUFA is provided (Trushenski et al. 2011, 2012). In both trials a reduction of dietary LC-PUFA was connected with reduced levels of the respective fatty acids in tissues. The trend of decreased LC-PUFA content in tissues indicates an inability to transform the 18-carbon precursors (ALA and LA) to their respective LC-PUFA (DHA, EPA, and ARA, respectively).

The lack of 20:3n-9, and subsequently the ratio of 20:3n-9:20:4n-6, appears to further substantiate the claim of Florida Pompano's inability to elongate and desaturate C_{18} PUFA to LC-PUFA.

Formation of 20:3n-9 requires Δ5 desaturation (Tocher 2010), the same process that is required in the formation of EPA and ARA in their formation from ALA and LA, respectively. The formation of 20:3n-9 from 18:1n-9 has been shown in previous studies to occur when a limiting amount of LA is provided to both mice and rats (Allmann and Gibson 1965; Ling et al. 2012). Although the diets all contained a low level of LA, fish exhibited no capacity to produce ARA or 20:3n-9 from their respective C_{18} PUFA. Although successful elongation and desaturation of C_{18} PUFA would also result in very low levels of 20:3n-9, limited availability of 18:1n-9 in all diets may have constrained any limited amount of synthesis occurring. Combining the lack of 20:3n-9 with the low levels of ARA in all diets lacking supplementation indicate that Florida Pompano are unable to properly form LC-PUFA do novo. If the capacity to form LC-PUFA was present, fish fed diets containing LA or ALA would be expected to exhibit tissue LC-PUFA levels higher than those fed the EFA Free (-) Control, if not similar to those fed diets containing intact LC-PUFA. Provision of LA and/or ALA did not appear to result in significant increases in tissue levels of ARA, EPA, or DHA (particularly in liver tissues, the primary site of LC-PUFA biosynthesis) as observed in Nile Tilapia (Chapter 2), suggesting that *de novo* synthesis was absent or limited in Florida Pompano.

In contrast to 20:3n-9, the presence of 22:5n-6 was noted in all tissues. The formation of 22:5n-6 utilizes Δ6 desaturation after the formation of ARA, similar to how EPA is used in the formation of DHA. If EPA is limiting, DHA synthesis will be reduced and transformation of the next preferred substrate for Δ6 desaturase, namely ARA, will increase. Neuringer et al. (1986) provided pregnant rhesus monkeys, and their offspring, with diets deficient in both DHA and ALA. In all offspring provided with a deficient diet, a significant increase in 22:5n-6 was reported across brain and retinal tissue. Even though no level of 22:5n-6 was specified as indicating deficiency, the offspring with elevated 22:5n-6 levels did exhibit poorly developed neural and ocular systems. Although my trial resulted in significant differences in levels of 22:5n-6, no differences in behavior or survival were noted. The absence of these gross indicators of EFA deficiencies indicates that the fish had not reached the critical threshold for developmental inhibition. A longer trial might have yielded greater evidence of deficiency, including gross pathology or mortality.

Florida Pompano, a marine carnivore (trophic level 3.5) (Froese and Pauly 2016), do not have a defined fatty acid requirement. Based on the results of my trial, there are indications that Florida

Pompano lack that ability to properly synthesize LC-PUFA from C_{18} PUFA. When provided with diets containing intact LC-PUFA, there was numerically superior growth and tissue fatty acid compositions that were more closely similar to the Fish Oil Control. The provision of dietary LC-PUFA has been shown to restore growth performance and tissue fatty acid composition in other carnivorous species including: European Seabass (*Dicentrarchus labrax*), Cobia (*Rachycentron canadum*), and Red Drum (*Sciaenops ocellatus*) (Craig and Gatlin 1995; Mourente et al. 2006; Trushenski et al. 2011, 2012). Although there was similarity between diets containing LC-PUFA and the positive Fish Oil Control, the levels of LC-PUFA available in the experimental diets were different than that available in the positive control (Table 3.3). The amount of ARA in both ARA+DHA and LC-PUFA diets was more than 4 times that provided by the Fish Oil Control, however, the levels of both EPA and DHA were less than one half of that of the positive control. Although there is no established EFA requirement for Florida Pompano, my study indicates that the levels of fatty acids (Table 3.3) provided are sufficient for various species of fish with established EFA requirements. Tocher (2010) assembled a variety of fish species of different salinity and temperature tolerances, with many of the marine species displaying EFA requirements of 0.3 – 5.5 % of the dry diet. Results from my trial indicate that Florida Pompano have superior performance when provided with at least 5.5 g fatty acid/kg of diet (0.55% dry diet) or when provided with 9.9 g fatty acid/kg of diet (approximately 1.0% dry diet) of ARA and DHA combined. These results are similar to that reported for European Seabass (*Dicentrarchus labrax*; trophic level 3.47) (Froese and Pauly 2016) which has a reported EFA requirement of 1.0 % [dry diet] n-3 LC-PUFA (NRC 2011). Other carnivorous species exhibit similar dietary requirements for LC-PUFA including: Atlantic Salmon (*Salmo salar;* trophic level = 3.76) (Froese and Pauly 2016), Gilthead Sea Bream (*Sparus aurata;* trophic level = 3.4) (Froese and Pauly 2016), Red Sea Bream (*Pagrus major;* trophic level = 3.7) (Froese and Pauly 2016), Striped Jack (*Pseudocaranx dentex;* trophic level = 3.66) (Froese and Pauly 2016), and Yellowtail (*Seriola quinqueradiata;* trophic level = 3.96) (Froese and Pauly 2016). Cumulatively, these species combined with the results of my trial indicate that species of a higher trophic level, regardless of salinity of thermal preference, require dietary LC-PUFA in order to meet their physiological demands. This information can be useful in the determination of a potential aquaculture species' fatty acid requirements.

Although non-significant, numerical differences between dietary treatments indicate better performance, which, combined with tissue fatty acid composition provide evidence for an LC-PUFA requirement for Florida Pompano. This conclusion supports my hypothesis that Florida Pompano are similar to other carnivorous species in their dietary requirement for LC-PUFAs. If my trial was conducted for a longer period of time these numerical differences might have developed into significant differences. Additional trials are required to determine specific values that juvenile Florida Pompano require for optimal growth and fatty acid composition, followed by advanced stages of growth and reproductive status. Future studies with other carnivorous species will provide additional information as to the use of trophic level as a successful indicator of fatty acid requirements.

CHAPTER 4

RECOMMENDATIONS FOR DIETARY FATTY ACID INCLUSION BASED ON TROPHIC LEVEL

Nile Tilapia exhibited the ability to utilize C_{18} PUFA in the proper formation of LC-PUFA (i.e., tissue profiles suggest that some of the ALA and LA provided in the LC-PUFA-free experimental diets was transformed into their n-3 and n-6 LC-PUFA derivatives), and no evidence of essential fatty acid deficiency was observed in fish provided diets containing LA and ALA. The successful use of C_{18} PUFA-rich ingredients in diets for Nile Tilapia has been shown previously (Chou and Shiau 1996; Teshima 1982; Trushenski et al. 2009; Mulligan and Trushenski 2013), and is not surprising as it occupies a low trophic level (2.1) (Froese and Pauly 2016). The natural diet of Nile Tilapia consists largely of detritus and plant material, both of which are likely to contain large amounts of C_{18} PUFA rather than LC-PUFA. As LC-PUFA biosynthesis is energetically costly, it was hypothesized that dietary inclusion of LC-PUFA would result in more efficient growth. Tissue levels of ARA, DHA and EPA were significantly higher in most tissues when these LC-PUFAs were supplemented directly in the diet, but growth was not significantly enhanced. Indeed, the fatty acid deficiency indicator ratios (20:3n-9/20:4n-6 and 22:5n-6/22:6n-3) suggested that direct provision of n-3 LC-PUFA might inhibit synthesis of n-6 LC-PUFA: a high 20:3n-9/20:4n-6 ratio in the DHA treatment raises concern regarding the possibility of a developing ARA deficiency. It has been established in other species that dietary provision of LC-PUFA results in suppression of elongase and/or desaturase activity (Clarke 2001; Ling et al. 2012) in those species capable of *de novo* LC-PUFA synthesis. Given that the same enzymes are involved in synthesis of both n-3 and n-6 LC-PUFA, it is possible that excessive dietary supplementation with DHA (and potentially EPA) may effectively induce an ARA deficiency. When LA or ARA were included into dietary treatments, the levels of 20:3n-9/20:4n-6 were reduced, supporting the LA requirement previously reported (NRC 2011); however, when LA was provided in the absence of ALA, (i.e., EFA Free (-) Control), Nile Tilapia exhibited significantly higher values of 22:5n-6/22:6n-3. This elevated level of the 22:5n-6/22:6n-3 ratio indicates a developing DHA deficiency, supporting previous claims made for the inclusion of both n-3 and n-6 fatty acids in the related hybrid Tilapia (*Oreochromis niloticus* ♀ x O. *aureus* ♂) (Chou 1999). Despite desirable effects of dietary LC-PUFA supplementation on the fatty acid composition of the edible tissues,

this study clearly demonstrated that Nile Tilapia can synthesize LC-PUFA from C_{18} PUFA in order to satisfy physiological demand and that dietary supplementation with DHA, in the absence of ARA, might lead to an n-6 LC-PUFA deficiency in this species.

Based on production performance results, one might conclude that the essential fatty acid requirements of Florida Pompano are 1) effectively met by either C_{18} PUFA or LC-PUFA, or 2) this species does not require any PUFA whatsoever. The lack of significant differences in growth performance between the EFA-free (-) Control and various experimental treatments mask what I believe to be developing essential fatty acid deficiencies in at least some of these treatment groups. First, growth performance was numerically, if not statistically, improved among fish fed the C_{18} PUFA supplemented feeds and further improved among fish fed combinations of LC-PUFAs. It is possible that a longer feeding trial would have allowed these potential treatment effects to develop into statistical significance. Second, the fatty acid deficiency indicator ratios seem to suggest that essential fatty acid deficiency was developing in at least some of the dietary treatment groups. Although the absence of 20:3n-9 could be indicative of a capacity to synthesize LC-PUFA, it is equally plausible that the limited amount of 18:1n-9 in the diets limited synthesis. Elevated levels of the 22:5n-6/22:6n-3 ratio in both ARA+DHA and LC-PUFA treatments suggest there is some EFA limitation, but the elevated indicator ratios being observed in the ARA+DHA and LC-PUFA treatment groups is puzzling. In contrast to the low trophic level of Nile Tilapia, Florida Pompano occupy a much higher trophic position (trophic level = 3.5) (Froese and Pauly 2016), with much of their diet consisting largely of crustaceans and other benthic invertebrates. Based on their higher trophic level, I predicted that Florida Pompano lack the capacity to transform C_{18} PUFA into LC-PUFA. Although there were no significant differences between any experimental treatment and the EFA-Free control, performance was numerically superior among fish fed the ARA+DHA and LC-PUFA treatments. Of course, this conclusion is somewhat undercut by the observation of elevated 22:5n-6/22:6n-3 ratios in these same treatments. Nonetheless, provision of LA and/or ALA did not appear to result in significant increases in tissue levels of ARA, EPA, or DHA (particularly in liver tissues, the primary site of LC-PUFA biosynthesis) as observed in Nile Tilapia, suggesting that *de novo* synthesis was absent or limited in Florida Pompano. The requirement for LC-PUFA has been shown in many other species (Williams et al. 1985; Nematipour and Gatlin 1993; Craig and Gatlin 1995; Xu and Kestemont

2002; Luzzana et al. 2003; Mourente et al. 2006; Trushenski et al. 2011, 2012), most of these occupying similar or higher trophic levels compared to Florida Pompano. Tissue fatty acid composition was consistent with results of the Nile Tilapia study, with fish not provided with LC-PUFA in the diet (i.e., fish fed the ALA, C_{18} PUFA, and EFA Free Control feeds) exhibiting significantly lower levels of ARA, EPA, and DHA in comparison with fish fed these nutrients directly.

My results indicate that Nile Tilapia can utilize C_{18} PUFA to synthesize LC-PUFA, and both n-3 and n-6 PUFA are required to meet the essential fatty acid requirements of Nile Tilapia. Other species with similar trophic levels including Grass Carp (freshwater, coolwater, trophic level = 2.0) (Froese and Pauly 2016), Milkfish (marine, tropical, trophic level = 2.4) (Froese and Pauly 2016), Common Carp (freshwater, coolwater, trophic level = 3.05) (Froese and Pauly 2016) and Channel Catfish (freshwater, coolwater, trophic level = 3.4) (Froese and Pauly 2016) all reportedly require C_{18} PUFA, but not LC-PUFA (NRC 2011). These species represent both freshwater and marine guilds and a variety of preferred thermal ranges, but similar fatty acid requirements. Additional studies must be conducted to quantitatively determine specific fatty acid requirements for individual species, but in the absence of such information, trophic levels may be a useful predictor of C_{18} PUFA vs. LC-PUFA essentiality and beneficial for guiding nutritional studies.

Further my results indicate that Florida Pompano have little-to-no capacity for LC-PUFA synthesis regardless of C_{18} PUFA availability. A requirement for dietary LC-PUFA has been reported in other carnivorous species including Striped Bass (anadromous, coolwater, trophic level = 4.04) (Froese and Pauly 2016), Atlantic Salmon (anadromous, coldwater, trophic level = 3.76) (Froese and Pauly 2016), Mahi Mahi (marine, warmwater, trophic level = 4.48) (Froese and Pauly 2016), Striped Jack (*Pseudocaranx dentex*; marine, warmwater, trophic level = 3.66) (Froese and Pauly 2016) and Yellowtail (*Seriola quinqueradiata;* marine, coolwater, trophic level = 3.96) (Froese and Pauly 2016) (NRC 2011). As noted for C_{18} PUFA requirements among lower trophic level species, requirements for LC-PUFA are not limited to freshwater or marine fish or species that prefer warm, cool, or cold water. As noted above, many individual studies may be needed to quantitatively determine taxonomically specific EFA requirements in untested species; however, one can expect all species to exhibit requirements for both n-

3 and n-6 PUFA and trophic level appears to be a reliable indicator of C_{18} PUFA vs. LC-PUFA essentiality.

The goal of this research was to assess the essentiality of C_{18} PUFA or LC-PUFA in feeds for taxa representing freshwater/marine and omnivore/carnivore dichotomies, address the relative utility of thermal vs. salinity vs. trophic guilds as predictors of PUFA essentiality; and, if appropriate, facilitate better use of trophic level as predictor of fatty acid requirements of fish. In the course of my work, I demonstrated that both n-3 and n-6 PUFA are needed in the diets of Nile Tilapia and Florida Pompano. Further, I demonstrated that whereas LC-PUFA are unnecessary, perhaps even detrimental in feeds for Nile Tilapia, direct dietary provision of LC-PUFA may be beneficial, if not necessary, for Florida Pompano. Given these differential results and the trophic levels of the species involved, it appears best to use trophic level as a predictor of LC-PUFA essentiality in lieu of preferred water temperatures or salinities.

Table 2.1. Feed formulations for Nile Tilapia (based on previously validated feed formulations; Trushenski et al. 2009 and Mulligan and Trushenski 2013). All values expressed as g/kg feed.

Ingredient (g/kg)	Fish Oil (+) Control	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
Menhaden fish meal[1]	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Soybean meal[1]	422.0	422.0	422.0	422.0	422.0	422.0	422.0
Wheat bran	182.8	182.8	182.8	182.8	182.8	182.8	182.8
Corn gluten meal	180.0	180.0	180.0	180.0	180.0	180.0	180.0
Fish oil	51.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydrogenated soybean oil	0.0	51.0	31.0	41.0	46.0	41.0	36.0
Hydrogenated soybean lecithin	5.0	5.0	5.0	5.0	5.0	5.0	5.0
18:2n-6 ethyl ester	0.0	0.0	0.0	10.0	0.0	0.0	0.0
20:4n-6 ethyl ester	0.0	0.0	0.0	0.0	0.0	5.0	5.0
18:3n-3 ethyl ester	0.0	0.0	10.0	10.0	0.0	0.0	0.0
20:5n-3 ethyl ester	0.0	0.0	0.0	0.0	0.0	0.0	5.0
22:6n-3 ethyl ester	0.0	0.0	0.0	0.0	5.0	5.0	5.0
Sodium phosphate	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Dicalcium phosphate	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin premix	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay-C	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Carboxymethyl cellulose	20.0	20.0	20.0	20.0	20.0	20.0	20.0

[1] Solvent extracted.

[2] Estimates are based on purified fatty acid ethyl ester supplements and inclusion rates and fatty acid composition of menhaden fish oil, hydrogenated soybean oil, hydrogenated soybean lecithin and a fatty acid/crude lipid weight conversion factor of 0.93 (Weihrauch et al. 1977). Trace lipid content of other practical ingredients was not included in these calculations.

[3] Fatty acid supplements included in LC-PUFA diet include ARA, DHA, and EPA

Table 2.2. Proximate and fatty acid composition (means ± SE) of Nile Tilapia diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) [1]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
Proximate composition			*g/kg, dry matter basis (except Dry Matter)*				
Dry Matter	971 ± 0	978 ± 0	957 ± 18	966 ± 7	966 ± 0	962 ± 1	971 ± 2
Protein	447 ± 12	441 ± 1	442 ± 2	473 ± 1	438 ± 2	440 ± 3	440 ± 2
Lipid	88 ± 1	79 ± 1	83 ± 1	83 ± 2	84 ± 1	86 ± 1	88 ± 1
Ash	96 ± 0	95 ± 1	96 ± 0	109 ± 0	96 ± 1	95 ± 0	96 ± 0
Fatty acid(s)			*g/100 g Fatty Acid Methyl Esters (FAME)*				
14:0	5.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	17.5 ± 0.2	13.9 ± 0.0	12.3 ± 0.1	11.0 ± 0.0	13.1 ± 0.0	12.2 ± 0.0	11.4 ± 0.0
18:0	7.7 ± 0.1	61.7 ± 0.1	52.4 ± 0.4	41.1 ± 0.0	57.0 ± 0.2	51.5 ± 0.5	46.1 ± 0.1
SFA[a]	32.3 ± 0.4	77.1 ± 0.2	66.1 ± 0.3	53.4 ± 0.1	71.4 ± 0.2	65.1 ± 0.4	58.6 ± 0.1
16:1	7.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:1n-7	2.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:1n-9	9.2 ± 0.1	5.2 ± 0.0	5.1 ± 0.1	5.1 ± 0.0	5.1 ± 0.0	5.1 ± 0.1	5.0 ± 0.0
MUFA[b]	19.8 ± 0.2	6.0 ± 0.0	5.8 ± 0.1	6.0 ± 0.0	5.9 ± 0.0	5.8 ± 0.1	5.8 ± 0.0
18:2n-6	17.1 ± 0.1	14.8 ± 0.1	15.2 ± 0.2	26.1 ± 0.1	14.7 ± 0.1	14.9 ± 0.3	14.9 ± 0.0
20:4n-6	0.8 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	6.0 ± 0.1	6.4 ± 0.0
n-6[c]	18.6 ± 0.1	14.8 ± 0.1	15.2 ± 0.2	26.2 ± 0.0	14.7 ± 0.1	20.9 ± 0.3	21.3 ± 0.1
18:3n-3	2.7 ± 0.0	1.5 ± 0.0	12.3 ± 0.1	13.1 ± 0.1	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.0
18:4n-3	2.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:4n-3	1.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:5n-3	9.2 ± 0.2	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	6.3 ± 0.0
22:5n-3	1.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6n-3	10.4 ± 0.3	0.4 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	6.4 ± 0.0	6.5 ± 0.1	6.4 ± 0.0
n-3[d]	27.3 ± 0.7	2.1 ± 0.0	12.9 ± 0.1	14.4 ± 0.1	8.1 ± 0.0	8.2 ± 0.1	14.3 ± 0.1
PUFA[e]	47.9 ± 0.6	16.9 ± 0.1	28.1 ± 0.3	40.6 ± 0.0	22.8 ± 0.1	29.1 ± 0.3	35.6 ± 0.1
C_{18} PUFA[f]	22.7 ± 0.1	16.4 ± 0.1	27.5 ± 0.3	39.2 ± 0.0	16.2 ± 0.1	16.5 ± 0.3	16.5 ± 0.0
LC-PUFA[g]	23.5 ± 0.6	0.5 ± 0.0	0.6 ± 0.0	1.4 ± 0.0	6.6 ± 0.0	12.6 ± 0.2	19.0 ± 0.1

Table 2.2. Proximate and fatty acid composition (means ± SE) of Nile Tilapia diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) [1]

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 15:0, 17:0, 20:0, 22:0, 21:0, 22:0, 23:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond; include 14:1, 15:1, 17:1, 20:1n-9, 22:1n-11, 22:1n-9 and 24:1n-9 in addition to individually reported MUFA

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 16:2n-4, 16:3n-4, 18:2n-6 tans, 18:3n-6, 18:3n-4, 20:2, 20:3n-9, 20:3n-6, 22:2 and 22:5n-6 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

Table 2.3. Fatty acid composition of Nile Tilapia feeds (g fatty acid/kg feed); Reported Requirement (NRC 2011) adjusted from % dry diet to g fatty acid/kg feed

	Reported Requirement	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
18:2n-6	5.0	14.0	10.9	11.7	20.1	11.5	11.9	12.2
20:4n-6	n/a	0.7	0.0	0.0	0.0	0.0	4.8	5.2
18:3n-3	n/a	2.2	1.1	9.5	10.1	1.2	1.3	1.3
20:5n-3	n/a	7.5	0.1	0.2	0.2	0.0	0.2	5.2
22:6n-3	n/a	8.5	0.3	0.4	0.7	5.0	5.2	5.2
C18 PUFA	n/a	16.2	12.0	21.2	30.3	12.7	13.2	13.5
n-3 LC-PUFA	n/a	16.0	0.4	0.5	0.9	5.0	5.4	10.4
Total LC-PUFA	n/a	16.7	0.4	0.5	1.0	5.0	10.2	15.6

Table 2.4. Production performance of Nile Tilapia fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)

Parameter	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Survival (%)	97.5	100	95	100	92.5	97.5	97.5	3.1	0.227
Initial weight (g)	25.8	25.7	25.6	25.6	25.7	25.9	25.7	0.1	0.605
Final weight (g)	134.3 z	108.6 y	117.3 zy	125.6 zy	112.0 y	113.0 y	119.5 zy	6.2	0.007
Weight gain (%)	421.4 z	322.8 y	358.2 zy	390.6 zy	335.5 y	337.2 y	365.5 zy	23.6	0.006
SGR (% body weight/day)	3.3 z	2.9 y	3.0 zy	3.2 zy	2.9 y	2.9 y	3.1 zy	0.1	0.007
FCR (dry matter basis)	1.0 y	1.2 z	1.2 z	1.1 zy	1.1 z	1.2 z	1.2 z	0.0	0.001
Feed Intake (% body weight/day)	3.6	3.6	3.9	3.8	3.7	3.7	3.9	0.2	0.308
HSI	0.9	0.8	0.8	0.8	0.9	0.9	1.0	0.1	0.672
LSI	6.2	6.5	6.1	6.4	10.0	6.6	6.9	1.7	0.300

Table 2.5. Fatty acid composition of fillet tissue of Nile Tilapia fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
14:0	3.0 z	1.8 y	1.7 y	1.8 y	1.7 y	1.5 y	1.6 y	0.2	< 0.001
16:0	21.5	23.9	22.2	21.8	21.9	22.2	21.3	0.9	0.122
18:0	9.7 x	11.3 zy	11.7 zy	10.5 yx	11.5 zy	12.1 z	11.8 zy	0.4	< 0.001
SFA[a]	35.2 y	38.0 z	36.3 zy	34.8 y	36.0 zy	36.6 zy	35.4 zy	0.8	0.015
16:1	4.0 z	2.3 y	2.0 y	2.4 y	1.9 y	2.0 y	2.0 y	0.4	< 0.001
18:1n-7	3.7 y	3.9 z	3.1 wv	2.9 v	3.4 x	3.2 xw	2.9 v	0.1	< 0.001
18:1n-9	10.4	13.2	13.4	13.5	13.7	12.1	12.9	1.6	0.452
MUFA[b]	19.2	20.8	19.6	19.9	21.6	18.5	19.0	2.1	0.783
18:2n-6	9.2 w	12.0 y	11.7 y	16.2 z	11.5 yx	9.9 xw	9.6 w	0.5	< 0.001
20:3n-6	0.7 x	1.4 z	1.2 zy	1.3 zy	1.1 y	0.7 x	0.6 x	0.1	< 0.001
20:4n-6	2.3 x	5.3 y	3.7 x	3.6 x	3.1 x	8.2 z	7.7 z	0.5	< 0.001
22:5n-6	0.9 w	3.9 z	1.7 x	1.9 x	1.6 x	2.6 y	1.7 x	0.1	< 0.001
n-6[c]	13.4 w	23.3 z	18.9 x	23.7 z	17.7 x	21.8 zy	20.0 yx	0.8	< 0.001
18:3n-3	1.1 y	0.7 y	5.4 z	5.3 z	0.8 y	0.8 y	0.8 y	0.2	< 0.001
20:5n-3	2.8 z	0.9 xw	1.1 x	0.8 xw	0.8 xw	0.7 w	1.9 y	0.1	< 0.001
22:5n-3	5.4 z	2.2 wv	2.9 x	2.3 xw	1.5 vu	1.5 u	4.0 y	0.2	< 0.001
22:6n-3	20.3 z	11.9 x	13.8 yx	11.2 x	19.9 z	18.7 zy	17.8 zy	1.6	< 0.001
n-3[d]	30.9 z	15.8 x	23.7 y	19.9 yx	23.2 y	21.7 y	24.5 y	1.8	< 0.001
20:3n-9	0.1 x	0.6 z	0.3 zyx	0.3 yx	0.4 zy	0.2 yx	0.2 yx	0.1	< 0.001
PUFA[e]	45.6	41.2	44.1	45.2	42.5	44.9	45.6	2.4	0.451
C_{18} PUFA[f]	11.3 wv	13.6 x	17.9 y	22.4 z	12.9 xw	11.1 wv	10.9 v	0.6	< 0.001
LC-PUFA[g]	33.2 zy	26.3 yx	25.1 x	21.6 x	28.5 zyx	32.7 zy	33.9 z	2.3	< 0.001
n-3:n-6	2.3 z	0.7 v	1.3 yx	0.8 wv	1.3 y	1.0 xw	1.2 yx	0.1	< 0.001
20:3n9 : 20:4n-6	0.036 y	0.127 z	0.095 zy	0.079 zy	0.143 z	0.026 y	0.026 y	0.024	< 0.001
22:5n-6 : 22:6n-3	0.043 w	0.353 z	0.128 yx	0.180 y	0.083 xw	0.148 yx	0.101 yxw	0.024	< 0.001

Table 2.5, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 15:0, 17:0, 20:0, 22:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 14:1, 15:1, 16:1, 16:2n-4, 16:3n-4, 17:1, 18:3n-6, 18:3n-4, 18:4n-3, 20:1n-9, 20:2, 20:3n-6, 20:4n-3, 22:1n-9 and 24:1n-9 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

Table 2.6. Fatty acid composition of liver tissue of Nile Tilapia fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
14:0	2.8 z	2.1 zy	1.8 y	1.8 y	1.7 y	1.7 y	1.6 y	0.2	0.001
16:0	21.6	23.2	22.1	22.0	23.0	21.8	21.4	0.9	0.329
18:0	14.1	16.8	16.2	16.7	16.4	16.7	16.5	1.1	0.239
SFA[a]	39.8	43.1	41.1	41.5	42.2	41.2	40.5	1.6	0.486
16:1	3.6 z	2.2 y	1.6 y	1.9 y	1.7 y	1.9 y	1.7 y	0.3	< 0.001
18:1n-7	2.9 z	3.0 z	2.2 y	2.3 y	2.4 y	2.4 y	2.3 y	0.1	< 0.001
18:1n-9	10.2	10.7	10.4	11.1	10.8	9.7	10.1	1.1	0.900
MUFA[b]	17.9	17.0	15.3	16.3	16.1	15.2	15.2	1.4	0.390
18:2n-6	6.7 zy	6.3 zy	7.0 zy	8.3 z	5.6 zy	5.2 y	5.0 y	0.8	0.010
20:3n-6	0.8 y	1.5 z	1.3 z	1.5 z	1.2 z	0.8 y	0.8 y	0.1	< 0.001
20:4n-6	3.9 v	9.1 zy	7.2 xw	7.8 yx	6.3 w	9.6 z	9.2 z	0.4	< 0.001
22:5n-6	0.9 w	6.9 z	2.7 x	3.5 yx	2.6 x	4.4 y	3.0 yx	0.5	< 0.001
n-6[c]	12.6 v	24.3 z	18.5 x	21.5 y	16.1 w	20.3 yx	18.2 xw	0.1	< 0.001
18:3n-3	0.7 y	0.3 y	2.4 z	1.9 z	0.4 y	0.4 y	0.4 y	0.2	< 0.001
20:5n-3	1.5 z	0.2 x	0.6 yx	0.4 yx	0.3 x	0.2 x	0.7 y	0.1	< 0.001
22:5n-3	3.7 z	1.0 w	1.9 yx	1.4 xw	0.7 w	0.8 w	2.3 y	0.2	< 0.001
22:6n-3	21.8 z	12.1 x	18.3 yx	15.2 yx	22.5 z	20.5 z	21.5 z	1.5	< 0.001
n-3[d]	28.4 z	13.6 w	23.5 zyx	19.2 x	23.9 zyx	22.0 yx	25.0 zy	1.8	< 0.001
20:3n-9	0.2 x	1.0 z	0.6 zyx	0.4 yx	0.8 zy	0.4 yx	0.4 yx	0.1	< 0.001
PUFA[e]	42.3	39.9	43.6	42.2	41.7	43.6	44.4	2.1	0.445
C_{18} PUFA[f]	2.3 z	0.6 v	1.3 yx	0.9 w	1.5 y	1.1 xw	1.4 yx	0.1	< 0.001
LC-PUFA[g]	33.2 zyx	31.8 yx	32.8 zyx	30.4 x	34.5 zyx	36.8 zy	37.9 z	1.8	0.006
n-3:n-6	8.1 zyx	7.1 yx	9.8 zy	10.7 z	6.3 x	5.9 x	5.7 x	1.0	< 0.001
20:3n9 : 20:4n-6	0.044 x	0.109 zy	0.076 zyx	0.058 yx	0.126 z	0.045 x	0.040 x	0.018	< 0.001
22:5n-6 : 22:6n-3	0.042 x	0.582 z	0.152 yx	0.237 y	0.119 yx	0.221 y	0.143 yx	0.039	< 0.001

Table 2.6, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 15:0, 17:0, 20:0, 22:0 and 24:0 in addition to individually reported SFA
[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond
[c] Sum of all n-6 fatty acids
[d] Sum of all n-3 fatty acids
[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 14:1, 15:1, 16:1, 16:2n-4, 16:3n-4, 17:1, 18:3n-6, 18:3n-4, 18:4n-3, 20:1n-9, 20:2, 20:3n-6,
20:4n-3, 22:1n-9 and 24:1n-9 in addition to individually reported PUFA
[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3
[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

Table 2.7. Fatty acid composition of intraperitoneal fat of Nile Tilapia fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
14:0	6.3 zy	6.5 z	4.8 zyx	4.2 x	5.0 zyx	4.6 yx	4.6 yx	0.6	0.002
16:0	22.2 x	27.4 z	24.1 yx	23.2 yx	25.7 zy	23.8 yx	22.4 x	1.0	0.003
18:0	8.2 x	13.0 z	11.5 zy	10.0 yx	11.7 zy	11.5 zy	10.8 zyx	0.9	< 0.001
21:0	0.7 y	1.3 y	1.0 y	0.9 y	0.9 y	3.2 z	3.1 z	0.2	< 0.001
SFA[a]	38.6 x	49.6 z	42.3 yx	39.1 x	44.3 y	44.2 y	41.8 yx	1.3	0.001
16:1	7.8 z	5.5 y	4.8 y	4.6 y	4.9 y	5.0 y	4.9 y	0.4	< 0.001
18:1n-7	3.6 zy	3.9 z	3.1 yx	2.9 x	3.5 zy	3.3 yx	3.1 yx	0.2	< 0.001
18:1n-9	15.4 y	18.4 zy	20.0 z	18.8 zy	20.2 z	18.9 zy	19.6 z	1.3	0.022
20:1n-9	1.0 x	1.8 z	1.4 zyx	1.2 yx	1.6 zy	1.4 zyx	1.4 zyx	0.1	< 0.001
22:1n-11	0.6	2.0	1.0	0.8	1.3	1.2	1.2	0.5	0.144
MUFA[b]	29.0	32.5	30.9	28.8	32.2	30.4	30.8	1.5	0.144
18:2n-6	12.1 y	10.1 y	11.7 y	17.7 z	12.7 y	13.0 y	12.5 y	1.2	< 0.001
20:4n-6	0.2 y	0.1 y	0.9 z	0.8 z	0.2 y	0.2 y	0.1 y	0.1	< 0.001
22:5n-6	0.3 w	0.6 zy	0.3 xw	0.4 yxw	0.4 xw	0.8 z	0.6 zyx	0.1	< 0.001
n-6[c]	13.5 y	12.0 y	14.0 y	20.4 z	14.2 y	14.9 y	14.1 y	1.3	< 0.001
18:3n-3	1.6 y	0.1 y	6.1 z	6.2 z	0.1 y	1.1 y	1.1 y	0.0	< 0.001
20:5n-3	1.7 z	0.3 x	0.4 x	0.3 x	0.3 x	0.4 x	1.2 y	0.1	< 0.001
22:5n-3	4.8 z	1.5 x	1.8 x	1.4 x	1.5 x	2.0 x	3.7 y	0.3	< 0.001
22:6n-3	7.1 z	1.7 x	2.5 x	2.1 x	4.7 y	5.3 y	5.6 zy	0.5	< 0.001
n-3[d]	17.0 z	4.4 w	11.4 y	10.4 yx	7.9 x	9.1 yx	12.0 y	1.0	< 0.001
20:3n-9	0.1 y	0.2 z	0.2 zy	0.2 zy	0.2 z	0.1 zy	0.2 zy	0.0	0.025
PUFA[e]	32.4 z	17.9 x	26.8 zy	32.2 z	23.5 yx	25.5 y	27.4 zy	2.0	< 0.001
C_{18} PUFA[f]	15.3 yx	11.5 x	18.7 y	24.9 z	14.4 yx	14.7 yx	14.3 yx	1.4	< 0.001
LC-PUFA[g]	15.6 z	5.1 v	7.0 xwv	6.1 wv	8.0 xw	9.5 yx	12.1 y	0.9	< 0.001
n-3:n-6	1.3 z	0.4 w	0.8 y	0.5 xw	0.6 x	0.6 x	0.9 y	0.0	< 0.001
20:3n9 : 20:4n-6	0.266 y	1.593 z	0.194 y	0.193 y	1.386 z	0.937 zy	1.344 z	0.312	< 0.001
22:5n-6 : 22:6n-3	0.043 t	0.378 z	0.134 xw	0.204 y	0.079 vt	0.153 x	0.103 wv	0.015	< 0.001

Table 2.7, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 15:0, 17:0, 20:0, 22:0 and 24:0 in addition to individually reported SFA
[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond
[c] Sum of all n-6 fatty acids
[d] Sum of all n-3 fatty acids
[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 14:1, 15:1, 16:1, 16:2n-4, 16:3n-4, 17:1, 18:3n-6, 18:3n-4, 18:4n-3, 20:1n-9, 20:2, 20:3n-6,
20:4n-3, 22:1n-9 and 24:1n-9 in addition to individually reported PUFA
[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3
[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

Table 2.8. Fatty acid composition of brain tissue of Nile Tilapia fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
14:0	4.4 z	2.4 y	2.4 y	2.6 y	2.5 y	2.6 y	2.3 y	0.3	< 0.001
16:0	21.8	23.3	21.9	22.0	22.5	22.6	21.4	0.7	0.246
18:0	11.2 y	14.2 z	13.8 zy	12.6 zy	13.3 zy	13.7 zy	13.8 zy	0.9	0.037
24:0	1.3	1.8	1.8	1.4	1.5	1.5	1.6	0.2	0.323
SFA[a]	39.9	42.9	40.9	39.5	40.8	41.5	40.1	1.4	0.328
16:1	6.4 z	3.9 y	3.7 y	4.0 y	3.8 y	4.2 y	3.6 y	0.3	< 0.001
18:1n-7	3.1 z	2.7 zy	2.4 y	2.5 y	2.7 zy	2.7 zy	2.4 y	0.1	< 0.001
18:1n-9	18.6 y	21.4 z	21.7 z	20.4 zy	21.4 z	21.1 z	20.5 zy	0.7	0.008
MUFA[b]	30.0	30.0	29.5	29.7	29.8	29.8	28.3	0.9	0.396
18:2n-6	8.5 y	7.4 y	7.6 y	12.4 z	9.1 y	9.3 zy	8.4 y	1.0	0.001
20:4n-6	1.2 w	2.8 y	2.1 x	1.8 xw	1.8 xw	3.6 z	3.8 z	0.2	< 0.001
22:5n-6	0.3 v	0.9 z	0.4 wv	0.5 xw	0.4 xwv	0.6 y	0.5 yx	0.0	< 0.001
n-6[c]	10.7 x	12.2 yx	11.0 yx	15.9 z	12.2 yx	14.2 zy	13.5 zyx	1.0	< 0.001
18:3n-3	1.1 y	0.6 y	4.2 z	4.3 z	0.8 y	0.8 y	0.8 y	0.3	< 0.001
20:5n-3	1.4 z	0.5 y	0.5 y	0.5 y	0.5 y	0.4 y	1.2 z	0.2	< 0.001
22:5n-3	3.5 z	1.3 y	1.6 y	1.4 y	1.4 y	1.3 y	2.8 z	0.2	< 0.001
22:6n-3	10.8 zy	11.2 zy	10.9 zy	8.1 y	13.0 z	10.7 zy	12.2 z	1.2	0.026
n-3[d]	18.0 z	13.8 zy	17.6 zy	14.8 zy	16.0 zy	13.4 y	17.3 zy	1.4	0.010
20:3n-9	0.1 y	0.2 z	0.1 zy	0.1 zy	0.2 z	0.1 zy	0.1 zy	0.0	0.001
PUFA[e]	30.2	27.1	29.5	31.8	29.4	28.6	31.6	1.9	0.227
C_{18} PUFA[f]	10.8 y	8.8 y	12.6 y	17.6 z	10.5 y	10.7 y	9.8 y	1.2	< 0.001
LC-PUFA[g]	18.3 zy	17.5 zyx	16.3 yx	13.3 x	18.0 zyx	17.2 zyx	21.2 z	1.5	0.003
n-3 : n-6	1.7 z	1.2 zy	1.6 z	1.0 y	1.5 zy	1.0 y	1.4 zy	0.20	< 0.001
20:3n9 : 20:4n-6	0.047 yx	0.109 zy	0.074 zyx	0.081 zyx	0.136 z	0.034 yx	0.030 x	0.024	0.002
22:5n-6 : 22:6n-3	0.025 w	0.101 z	0.035 xw	0.064 yx	0.033 w	0.071 zy	0.045 yxw	0.009	< 0.001

Table 2.8, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 15:0, 17:0, 20:0, 22:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 14:1, 15:1, 16:1, 16:2n-4, 16:3n-4, 17:1, 18:3n-6, 18:3n-4, 18:4n-3, 20:1n-9, 20:2, 20:3n-6, 20:4n-3, 22:1n-9 and 24:1n-9 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

Table 2.9. Fatty acid composition of eye tissue of Nile Tilapia fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)	*g/100 g Fatty Acid Methyl Esters (FAME)*								
14:0	5.7 z	4.7 y	4.0 x	3.6 wv	3.9 xw	3.7 xwv	3.5 v	0.1	< 0.001
16:0	19.1 x	23.2 z	20.3 yx	19.3 yx	21.3 zy	20.1 yx	18.9 x	0.6	< 0.001
18:0	6.4 w	8.1 y	8.6 zy	7.5 x	8.8 z	8.7 zy	8.5 zy	0.2	< 0.001
SFA[a]	32.2 xw	36.9 z	33.5 yx	31.1 w	34.8 zy	33.3 yx	31.7 xw	0.7	< 0.001
16:1	7.9 z	6.1 y	5.1 x	5.0 x	5.1 x	5.3 x	4.9 x	0.2	< 0.001
18:1n-7	3.5 zy	3.7 z	3.0 xw	2.8 w	3.3 yx	3.1 yxw	2.9 w	0.1	< 0.001
18:1n-9	15.0 y	20.3 z	19.7 z	18.1 zy	20.4 z	18.6 z	18.4 z	1.0	< 0.001
20:1n-9	0.9 y	1.3 z	1.1 zy	1.0 y	1.3 z	1.1 zy	1.0 y	0.1	< 0.001
MUFA[b]	28.2 y	32.4 z	29.9 zy	27.7 y	31.0 zy	29.0 zy	28.0 y	1.2	0.008
18:2n-6	12.9 y	16.2 y	15.2 y	20.6 z	16.5 y	16.6 y	15.0 y	1.2	< 0.001
20:4n-6	0.8 y	1.2 y	0.9 y	1.0 y	0.9 y	4.2 z	4.3 z	0.2	< 0.001
22:5n-6	0.3 w	0.7 y	0.3 w	0.5 x	0.4 xw	0.9 z	0.7 y	0.0	< 0.001
n-6[c]	15.1 w	20.0 zyx	17.8 xw	23.9 z	19.1 yxw	23.0 zy	21.2 zyx	1.3	< 0.001
18:3n-3	1.8 x	1.4 x	9.0 z	7.9 y	1.5 x	1.5 x	1.4 x	0.2	< 0.001
20:5n-3	2.6 z	0.9 x	0.9 x	0.8 x	0.7 x	0.9 x	2.2 y	0.1	< 0.001
22:6n-3	9.8 z	3.6 x	4.0 x	3.9 x	8.6 y	8.0 y	8.6 zy	0.4	< 0.001
n-3[d]	22.5 z	8.7 w	17.3 y	15.6 y	13.4 x	13.2 x	17.8 y	0.7	< 0.001
20:3n-9	0.1 y	0.3 z	0.2 zy	0.2 zy	0.3 z	0.2 zy	0.2 zy	0.0	< 0.001
PUFA[e]	39.6 zy	30.8 x	36.6 zyx	41.2 z	34.2 yx	37.7 zy	40.3 z	1.8	< 0.001
C_{18} PUFA[f]	16.7 y	19.2 y	25.6 z	30.1 z	19.0 y	19.1 y	17.4 y	1.4	< 0.001
LC-PUFA[g]	21.3 z	10.0 w	9.8 w	9.8 w	13.8 x	17.3 y	21.8 z	0.7	< 0.001
n-3 : n-6	1.5 z	0.4 u	1.0 y	0.7 wv	0.7 w	0.6 v	0.8 x	0.00	< 0.001
20:3n9 : 20:4n-6	0.074 yx	0.258 z	0.228 z	0.185 zy	0.305 z	0.042 x	0.039 x	0.041	< 0.001
22:5n-6 : 22:6n-3	0.036 v	0.203 z	0.086 xw	0.131 y	0.052 wv	0.120 yx	0.082 w	0.011	< 0.001

Table 2.9, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 15:0, 17:0, 20:0, 22:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 14:1, 15:1, 16:1, 16:2n-4, 16:3n-4, 17:1, 18:3n-6, 18:3n-4, 18:4n-3, 20:1n-9, 20:2, 20:3n-6, 20:4n-3, 22:1n-9 and 24:1n-9 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

Table 2.10. Coefficient of distance values of tissues of Nile Tilapia fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
Fillet	0.0	11.5	9.9	13.5	6.8	8.7	7.5
Liver	0.0	13.4	6.6	9.4	5.8	8.4	7.0
Brain	0.0	6.3	6.6	6.8	5.4	5.8	5.5
Eye	0.0	10.2	11.5	12.6	8.3	7.9	7.0
Intraperitoneal fat	0.0	8.6	8.3	9.2	7.8	6.7	6.5

Table 3.1. Feed formulations for Florida Pompano (based on previously validated feed formulations; Trushenski et al. 2011). All values expressed as g/kg feed.

Ingredient (g/kg)	Fish Oil (+) Control	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
Menhaden fish meal[1]	245.5	245.5	245.5	245.5	245.5	245.5	245.5
Soybean protein concentrate	137.0	137.0	137.0	137.0	137.0	137.0	137.0
Soy protein isolate	79.2	79.2	79.2	79.2	79.2	79.2	79.2
Wheat bran	277.3	277.3	277.3	277.3	277.3	277.3	277.3
Corn gluten meal	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Fish oil	72.7	0.0	0.0	0.0	0.0	0.0	0.0
Hydrogenated soybean oil	0.0	72.7	62.8	52.9	67.7	62.7	57.7
Hydrogenated soybean lecithin	5.0	5.0	5.0	5.0	5.0	5.0	5.0
18:2n-6 ethyl ester	0.0	0.0	0.0	9.9	0.0	0.0	0.0
20:4n-6 ethyl ester	0.0	0.0	0.0	0.0	0.0	5.0	5.0
18:3n-3 ethyl ester	0.0	0.0	9.9	9.9	0.0	0.0	0.0
20:5n-3 ethyl ester	0.0	0.0	0.0	0.0	0.0	0.0	5.0
22:6n-3 ethyl ester	0.0	0.0	0.0	0.0	5.0	5.0	5.0
Vitamin premix	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay-C	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Methionine chloride	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Choline chloride	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Carboxymethyl cellulose	20.0	20.0	20.0	20.0	20.0	20.0	20.0

[1] Solvent extracted.

[2] Estimates are based on purified fatty acid ethyl ester supplements and inclusion rates and fatty acid composition of menhaden fish oil, hydrogenated soybean oil, hydrogenated soybean lecithin and a fatty acid/crude lipid weight conversion factor of 0.93 (Weihrauch et al. 1977). Trace lipid content of other practical ingredients was not included in these calculations.

[3] LC-PUFA diet amended with ARA, DHA, and EPA

Table 3.2. Proximate and fatty acid composition (means ± SE) of Florida Pompano diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)[h]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
Proximate composition	*g/kg, dry matter basis (except Dry Matter)*						
Dry Matter	951.3 ± 0.0	963.3 ± 0.1	962.6 ± 0.3	959.1 ± 0.2	954.2 ± 0.2	954.2 ± 0.1	953.2 ± 0.2
Protein	571.9 ± 3.9	568.8 ± 3.0	566.9 ± 3.9	566.3 ± 2.7	568.6 ± 4.9	562.2 ± 2.8	566.0 ± 1.2
Lipid	108.4 ± 1.1	102.1 ± 0.8	104.1 ± 1.8	106.2 ± 0.6	103.4 ± 1.1	104.0 ± 1.0	105.1 ± 0.6
Ash	94.8 ± 1.0	95.3 ± 0.2	94.0 ± 0.9	95.7 ± 0.3	94.8 ± 0.3	95.7 ± 0.4	94.8 ± 0.2
Fatty acid(s)	*g/100 g Fatty Acid Methyl Esters (FAME)*						
14:0	6.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	17.8 ± 0.0	13.3 ± 0.0	12.1 ± 0.0	11.0 ± 0.0	12.7 ± 0.0	12.0 ± 0.0	11.5 ± 0.0
18:0	6.7 ± 0.0	70.0 ± 0.2	61.8 ± 0.2	53.1 ± 0.2	65.9 ± 0.3	61.7 ± 0.2	57.4 ± 0.0
SFA[a]	32.5 ± 0.2	84.7 ± 0.2	75.2 ± 0.2	65.2 ± 0.2	80.0 ± 0.3	75.1 ± 0.2	70.2 ± 0.1
16:1	8.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
18:1n-7	2.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:1n-9	8.6 ± 0.1	3.9 ± 0.1	3.9 ± 0.0	4.0 ± 0.0	3.9 ± 0.1	3.9 ± 0.1	4.0 ± 0.1
MUFA[b]	21.0 ± 0.0	4.6 ± 0.1	4.5 ± 0.1	4.7 ± 0.0	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.0
18:2n-6	12.3 ± 0.1	9.6 ± 0.1	9.9 ± 0.1	19.4 ± 0.0	9.6 ± 0.2	9.5 ± 0.1	9.8 ± 0.1
20:4n-6	0.9 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.9 ± 0.0	4.9 ± 0.0
n-6[c]	13.9 ± 0.1	9.6 ± 0.1	9.9 ± 0.1	19.4 ± 0.1	9.6 ± 0.2	14.4 ± 0.1	14.8 ± 0.1
18:3n-3	2.0 ± 0.0	0.7 ± 0.0	9.9 ± 0.0	10.1 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
18:4n-3	2.8 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:4n-3	1.3 ± 0.0	0.0 ± 00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:5n-3	10.6 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	4.7 ± 0.0
22:5n-3	1.8 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6n-3	11.9 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	4.9 ± 0.0	5.1 ± 0.0	5.0 ± 0.0
n-3[d]	30.4 ± 0.0	1.2 ± 0.0	10.4 ± 0.0	10.6 ± 0.0	5.7 ± 0.0	5.9 ± 0.0	10.5 ± 0.1
PUFA[e]	46.6 ± 0.1	10.8 ± 0.2	20.3 ± 0.1	30.1 ± 0.1	15.4 ± 0.2	20.4 ± 0.1	25.2 ± 0.0
C_{18} PUFA[f]	17.6 ± 0.1	10.3 ± 0.1	19.7 ± 0.1	29.5 ± 0.1	10.3 ± 0.2	10.2 ± 0.1	10.5 ± 0.1
LC-PUFA[g]	27.0 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	5.0 ± 0.0	10.1 ± 0.0	14.7 ± 0.1

Table 3.2, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 12:0, 13:0, 15:0, 17:0, 20:0, 21:0, 22:0, 21:0, 22:0, 23:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond; include 14:1, 15:1, 17:1, 20:1n-9, 22:1n-11, 22:1n-9 and 24:1n-9 in addition to individually reported MUFA

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 16:2n-4, 16:3n-4, 18:3n-6, 18:3n-4, 20:2, 20:3n-9, 20:3n-6, 22:2 and 22:5n-6 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[h] LC-PUFA diet amended with ethyl esters of DHA, ARA, and EPA

Table 3.3. Fatty acid content of Florida Pompano feeds (g fatty acid/kg feed); Reported Requirements (NRC 2011) for Florida Pompano currently undetermined (n/d).

	Reported Requirement	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
18:2n-6	n/d	12.4	9.1	9.6	19.2	9.2	9.2	9.6
20:4n-6	n/d	0.9	0.0	0.0	0.0	0.0	4.7	4.8
18:3n-3	n/d	2.0	0.7	9.6	10.0	0.7	0.7	0.7
20:5n-3	n/d	10.7	0.1	0.2	0.2	0.2	0.2	4.6
22:6n-3	n/d	12.0	0.3	0.3	0.4	4.7	4.9	4.9
C18 PUFA	n/d	14.4	9.8	19.2	29.1	9.9	9.9	10.3
n-3 LC-PUFA	n/d	22.7	0.4	0.5	0.6	4.9	5.1	9.5
Total LC-PUFA	n/d	23.6	0.4	0.5	0.6	4.9	9.9	14.3

Table 3.4. Production performance of Florida Pompano fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA). No mortalities were observed, therefore survival data were not analyzed (NA) using formal statistics.

Parameter	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Survival (%)	100	100	100	100	100	100	100	NA	NA
Initial weight (g)	47.6	47.4	47.2	47.3	46.7	47.7	47.7	0.5	0.400
Final weight (g)	153.8 z	117.2 y	121.2 y	124.3 y	119.8 y	130.1 y	130.1 y	4.0	< 0.001
Weight gain (%)	223.4 z	147.1 y	156.9 y	162.5 y	156.8 y	173.0 y	172.9 y	8.6	< 0.001
SGR (% body weight/day)	2.1 z	1.6 y	1.7 y	1.7 y	1.7 y	1.8 y	1.8 y	0.1	< 0.001
FCR (dry matter basis)	1.4 y	2.2 z	2.1 z	2.0 z	2.1 z	1.8 z	1.8 z	0.1	< 0.001
Feed Intake (% body weight/day)	3.1 y	3.6 z	3.6 z	3.5 z	3.6 z	3.4 z	3.4 z	0.1	< 0.001
HSI	1.8 zy	2.0 zy	2.2 z	2.1 zy	1.8 zy	1.6 y	1.6 y	0.2	0.009
LSI	5.1	6.0	5.7	6.0	5.6	5.3	5.1	0.5	0.294

Table 3.5. Fatty acid composition of fillet tissue of Florida Pompano fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)[h]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
14:0	7.1 z	4.3 y	4.0 y	3.8 y	4.2 y	3.7 y	3.7 y	0.3	< 0.001
16:0	28.0 x	32.0 z	30.9 zy	28.9 yx	32.9 z	32.1 z	31.3 z	0.7	< 0.001
18:0	6.1 x	6.6 yx	6.7 yx	6.2 x	7.3 zy	7.9 z	7.2 zy	0.2	< 0.001
SFA[a]	42.4 y	43.8 zy	42.5 y	39.7 x	45.4 z	44.6 zy	43.2 zy	0.7	< 0.001
16:1	8.5 z	4.3 y	3.8 yx	3.8 yx	3.8 yx	3.3 x	3.4 x	0.2	< 0.001
18:1n-7	3.0 z	2.4 y	2.1 x	1.9 x	2.3 yx	2.1 yx	2.1 x	0.1	< 0.001
18:1n-9	12.9 w	27.8 z	24.6 y	21.4 x	24.3 y	21.6 x	19.9 x	0.8	< 0.001
20:1n-9	0.6 w	1.7 z	1.2 yx	1.1 x	1.4 zy	1.3 yx	1.0 x	0.1	< 0.001
MUFA[b]	25.8 w	37.9 z	33.0 y	29.6 x	33.6 y	29.8 x	27.8 xw	0.8	< 0.001
18:2n-6	9.0 yxw	8.8 xw	9.7 yxw	16.3 z	8.4 w	10.3 yx	10.7 y	0.5	< 0.001
20:4n-6	0.6 x	0.3 w	0.3 w	0.2 w	0.3 xw	2.7 y	3.0 z	0.1	< 0.001
n-6[c]	10.2 x	9.5 x	10.3 x	16.9 z	9.1 x	13.8 y	14.4 y	0.5	< 0.001
18:3n-3	1.4 y	0.6 y	6.8 z	6.4 z	0.7 y	0.8 y	0.9 y	0.3	< 0.001
18:4n-3	1.4 z	0.2 y	0.2 y	0.3 y	0.3 y	0.2 y	0.3 y	0.0	< 0.001
20:4n-3	1.2 z	0.2 y	0.3 y	0.3 y	0.3 y	0.2 y	0.2 y	0.0	< 0.001
20:5n-3	4.0 z	1.0 x	0.8 x	0.9 x	1.0 x	0.9 x	2.5 y	0.1	< 0.001
22:5n-3	2.5 z	0.7 x	0.7 x	0.7 x	0.8 x	0.8 x	1.7 y	0.1	< 0.001
22:6n-3	9.6 z	5.0 y	4.6 y	4.1 y	8.0 z	8.0 z	8.5 z	0.6	< 0.001
n-3[d]	20.1 z	7.7 w	13.4 yx	12.7 yx	11.1 yx	11.0 x	14.0 y	0.9	< 0.001
PUFA[e]	31.8 z	18.3 v	24.5 xw	30.7 z	21.0 wv	25.5 yx	29.1 zy	1.0	< 0.001
C_{18} PUFA[f]	12.3 x	9.8 wv	16.8 y	23.1 z	9.5 v	11.5 yxw	11.9 xw	0.7	< 0.001
LC-PUFA[g]	18.3 z	7.5 x	6.9 x	6.4 x	10.7 y	13.3 y	16.5 z	0.9	< 0.001
n-3 : n-6	2.0 z	0.8 x	1.3 y	0.8 x	1.3 y	0.8 x	1.0 yx	0.1	< 0.001
20:3n9 : 20:4n-6	0.000	0.191	0.000	0.000	0.000	0.000	0.000	0.100	0.505
22:5n-6 : 22:6n-3	0.031 yx	0.045 zyx	0.031 yx	0.031 yx	0.020 x	0.068 z	0.051 zy	0.008	< 0.001

Table 3.5, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 17:0, 20:0, 22:0, 23:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond; include 14:1, 17:1, 20:1n-9, 22:1n-11 and 22:1n-9 in addition to individually reported MUFA

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 16:2n-4, 16:3n-4, 18:3n-6, 18:3n-4, 18:4n-3, 20:2, 20:3n-9, 20:3n-6, 20:4n-3 and 22:5n-6 in addition to individually reported PUFA

[f] C_{18} PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[h] LC-PUFA diet amended with ethyl esters of DHA, ARA, and EPA

Table 3.6. Fatty acid composition of liver tissue of Florida Pompano fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)[h]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
14:0	2.4	3.1	3.5	3.0	2.5	2.6	2.4	0.4	0.088
16:0	36.5 zy	31.0 x	32.2 yx	32.2 yx	38.0 z	36.1 zy	39.1 z	1.4	< 0.001
18:0	8.4 zy	8.3 zy	8.7 zy	7.8 y	10.6 zy	13.9 z	11.5 zy	1.6	0.020
SFA[a]	48.6 zyx	43.1 x	45.0 yx	43.5 x	51.9 zy	54.0 z	54.0 z	2.0	< 0.001
16:1	2.7 z	2.9 z	2.8 z	2.6 z	1.8 y	1.6 y	1.6 y	0.2	< 0.001
17:1	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.7	0.463
18:1n-7	3.9 z	2.4 y	2.1 yx	2.1 yx	1.6 y	1.6 yx	1.5 x	0.2	< 0.001
18:1n-9	15.4 x	38.6 z	36.8 z	34.4 zy	26.8 zyx	24.1 yx	23.5 yx	3.7	< 0.001
20:1n-9	1.4 y	3.4 z	3.2 z	3.1 z	2.4 zy	2.6 zy	2.2 zy	0.4	0.003
22:1n-9	0.5 y	1.0 z	1.0 z	0.9 zy	0.8 zy	0.9 z	0.7 zy	0.1	0.008
MUFA[b]	24.0 w	48.3 z	45.9 zy	43.2 zyx	33.5 yxw	32.2 yxw	29.4 xw	4.1	< 0.001
18:2n-6	5.0	4.4	4.1	7.6	5.2	2.8	3.6	1.8	0.233
20:4n-6	1.0 zy	0.1 y	0.1 y	0.2 y	0.3 y	2.6 z	2.8 z	0.7	0.002
n-6[c]	6.4	4.7	4.3	7.9	5.5	6.2	7.1	2.2	0.664
18:3n-3	0.4 zy	0.1 y	1.4 z	1.3 z	0.2 y	0.1 y	0.1 y	0.3	0.001
20:5n-3	1.5 z	0.1 y	0.1 y	0.1 y	0.2 y	0.1 y	0.5 y	0.2	< 0.001
22:5n-3	2.2 z	0.1 y	0.1 y	0.1 y	0.3 y	0.2 y	0.6 y	0.2	< 0.001
22:6n-3	14.5 z	2.9 y	2.3 y	2.3 y	7.4 y	6.5 y	7.3 y	1.8	< 0.001
n-3[d]	19.5 z	3.2 y	3.9 y	3.8 y	8.1 y	6.9 y	8.6 y	2.3	< 0.001
20:2	1.0 zy	0.6 y	0.6 y	1.3 z	0.7 zy	0.5 y	0.7 zy	0.2	0.007
PUFA[e]	27.4 z	8.6 y	9.0 y	13.2 zy	14.5 zy	13.8 zy	16.6 zy	4.4	0.015
C_{18} PUFA[f]	5.7	4.5	5.5	8.9	5.4	3.0	3.7	2.0	0.167
LC-PUFA[g]	20.4 z	3.3 y	2.7 y	2.8 y	8.2 y	10.2 y	12.0 zy	2.8	< 0.001
n-3 : n-6	3.0 z	0.7 wv	1.1 xwv	0.5 v	1.5 y	1.2 yxw	1.3 yx	0.1	< 0.001
20:3n9 : 20:4n-6	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]
22:5n-6 : 22:6n-3	0.023 x	0.038 x	0.029 x	0.027 x	0.011 x	0.152 z	0.106 y	0.01	< 0.001

Table 3.6, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 17:0, 20:0, 22:0, 23:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond; include 14:1 and 22:1n-11 in addition to individually reported MUFA

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 16:2n-4, 16:3n-4, 18:4n-3, 18:3n-4, 18:3n-6, 20:3n-6, 20:3n-9, 20:4n-3, 22:2, 22:5n-6 and 22:5n-3 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[h] LC-PUFA diet amended with ethyl esters of DHA, ARA, and EPA

[i] ND – Fatty acid not detected

Table 3.7. Fatty acid composition of intraperitoneal fat of Florida Pompano fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)[h]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
14:0	8.0 z	4.8 y	5.7 zy	4.7 y	5.4 y	4.5 y	5.7 zy	0.7	0.003
16:0	32.1 y	34.0 zy	36.5 zy	36.1 zy	36.1 zy	36.7 zy	37.6 z	1.5	0.043
18:0	6.9 y	7.8 y	7.3 y	7.8 y	8.2 zy	10.1 z	9.0 zy	0.7	0.004
SFA[a]	48.5 zy	47.8 y	50.7 zy	49.5 zy	51.0 zy	52.6 zy	53.5 z	1.5	0.016
16:1	8.3 z	4.3 z	4.8 y	4.1 y	4.5 y	3.7 y	4.7 y	0.6	< 0.001
18:1n-7	3.3 z	2.6 zy	2.5 zy	2.5 y	2.7 zy	2.4 y	2.7 zy	0.2	0.023
18:1n-9	13.9 y	26.0 z	20.7 z	22.8 zy	21.7 zy	21.7 zy	20.0 zy	2.4	0.008
20:1n-9	0.8 y	2.0 z	1.3 zy	1.6 zy	1.4 zy	1.6 zy	1.2 zy	0.3	0.051
22:1n-11	0.9	1.1	1.3	1.0	1.4	0.9	1.4	0.3	0.606
MUFA[b]	28.0 y	37.1 z	31.6 zy	32.7 zy	32.7 zy	31.2 zy	30.9 y	1.8	0.008
18:2n-6	7.6	8.0	7.5	9.3	7.5	6.8	6.5	1.1	0.289
20:4n-6	0.5 x	0.3 x	0.3 x	0.2 x	0.3 x	1.8 z	1.2 y	0.1	< 0.001
n-6[c]	8.6	8.6	8.1	9.7	8.1	9.1	8.1	1.1	0.707
18:3n-3	1.1 x	0.5 x	3.7 z	2.5 y	0.6 x	0.7 x	0.6 x	0.4	< 0.001
20:4n-3	1.0 z	0.3 y	0.4 y	0.3 y	0.3 y	0.3 y	0.3 y	0.1	< 0.001
20:5n-3	2.5 z	0.7 y	0.8 y	0.6 y	0.8 y	0.6 y	1.0 y	0.2	< 0.001
22:5n-3	2.0 z	0.6 y	0.8 y	0.6 y	0.8 y	0.7 y	1.0 y	0.2	< 0.001
22:6n-3	5.9 z	3.1 yx	2.9 yx	2.6 x	4.5 zy	4.0 yx	3.6 yx	0.5	< 0.001
n-3[d]	13.4 z	5.4 y	8.8 y	6.7 y	7.3 y	6.4 y	6.8 y	1.2	< 0.001
20:2	0.3 w	0.8 zy	0.6 yxw	1.0 z	0.6 yx	0.6 yxw	0.4 xw	0.1	< 0.001
PUFA[e]	23.5 z	15.1 y	17.7 zy	17.8 zy	16.4 zy	16.3 y	15.6 y	2.1	0.021
C_{18} PUFA[f]	10.0 z	8.8 z	11.5 z	12.0 z	8.5 z	7.7 z	7.5 z	1.5	0.039[i]
LC-PUFA[g]	12.3 z	5.3 y	5.3 y	4.4 y	6.9 y	7.8 y	7.4 y	1.0	< 0.001
n-3:n-6	1.6 z	0.6 x	1.1 y	0.7 x	0.9 zy	0.7 zy	0.8 zy	0.1	< 0.001
20:3n9 : 20:4n-6	0.119	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.463
22:5n-6 : 22:6n-3	0.037 y	0.035 y	0.030 y	0.019 y	0.020 y	0.079 z	0.044 zy	0.011	0.001

Table 3.7, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 17:0, 20:0, 22:0, 23:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond; include 14:1, 17:1 and 22:1n-9 in addition to individually reported MUFA

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 16:2n-4, 16:3n-4, 18:3n-6, 18:3n-4, 18:4n-3, 20:3n-9, 22:2, 20:3n-6 and 22:5n-6 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[h] LC-PUFA diet amended with ethyl esters of DHA, ARA, and EPA

[i] Despite significant findings from the omnibus ANOVA, pairwise comparisons failed to determine significant differences between means

Table 3.8. Fatty acid composition of brain tissue of Florida Pompano fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)[h]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)				*g/100 g Fatty Acid Methyl Esters (FAME)*					
16:0	25.8	25.1	26.2	26.2	25.5	26.7	25.5	1.4	0.911
18:0	14.9	14.9	14.2	15.3	15.2	16.1	15.7	0.9	0.501
24:0	2.2	2.3	2.1	1.9	2.3	2.1	2.4	0.3	0.721
SFA[a]	44.9	44.0	44.3	45.0	44.5	46.6	45.4	1.8	0.848
16:1	2.5 zy	2.4 zy	2.6 z	2.3 zy	2.1 zy	2.1 y	2.1 zy	0.2	0.029
18:1n-7	1.8 z	1.7 zyx	1.6 yx	1.6 x	1.6 yx	1.7 zyx	1.7 zy	0.0	0.004
18:1n-9	23.8	26.7	25.9	24.3	25.1	24.1	25.1	1.5	0.448
MUFA[b]	28.6	31.6	30.9	28.9	29.4	28.5	29.5	1.6	0.399
18:2n-6	1.1 w	2.5 y	2.4 yx	3.6 z	1.4 xw	1.5 yxw	1.2 w	0.3	< 0.001
20:4n-6	1.4 y	1.1 y	1.0 y	1.0 y	1.0 y	3.4 z	3.2 z	0.1	< 0.001
n-6[c]	2.8 x	4.4 y	4.0 y	5.4 z	2.7 x	5.5 z	4.9 zy	0.3	< 0.001
18:3n-3	0.1 y	0.1 y	0.9 z	0.6 z	0.0 y	0.1 y	0.0 y	0.1	< 0.001
20:5n-3	2.1 z	1.1 x	1.1 x	1.1 x	1.5 y	0.1 w	1.1 x	0.1	< 0.001
22:5n-3	1.6 z	1.0 yxw	0.9 yxw	0.9 xw	1.1 yx	0.8 w	1.1 y	0.1	< 0.001
22:6n-3	19.4	17.4	17.3	17.5	20.4	17.4	17.6	1.0	0.051
n-3[d]	23.6 z	19.7 yx	20.5 zyx	20.3 zyx	23.1 zy	19.2 x	20.0 zyx	1.1	0.006
PUFA[e]	26.5	24.4	24.8	26.1	26.1	24.9	25.1	1.2	0.510
C_{18} PUFA[f]	1.4 w	2.7 yx	3.4 zy	4.4 z	1.5 xw	1.7 xw	1.2 w	0.4	< 0.001
LC-PUFA[g]	25.0	21.3	21.1	21.3	24.4	23.1	23.7	1.3	0.584
n-3 : n-6	8.5 z	4.7 yx	5.2 y	3.8 x	8.7 z	3.5 x	4.1 yx	0.4	< 0.001
20:3n9 : 20:4n-6	0.000	0.017	0.000	0.000	0.022	0.000	0.014	0.000	0.584
22:5n-6 : 22:6n-3	0.009 y	0.017 zy	0.009 y	0.013 zy	0.009 y	0.027 z	0.024 z	0.004	0.002

Table 3.8, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 17:0, 20:0, 22:0 and 23:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond; include 14:1, 17:1, 20:1n-9, 22:1n-11 and 22:1n-9 in addition to individually reported MUFA

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 16:2n-4, 16:3n-4, 18:3n-3, 18:3n-6, 18:3n-4, 18:4n-3, 20:2, 20:3n-9, 20:3n-6, 20:4n-3, 22:2 and22:5n-6 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[h] LC-PUFA diet amended with ethyl esters of DHA, ARA, and EPA

Table 3.9. Fatty acid composition of eye tissue of Florida Pompano fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)[h]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA	Pooled SE	*P* Value
Fatty acid(s)	*g/100 g Fatty Acid Methyl Esters (FAME)*								
14:0	6.7 z	4.0 y	3.9 y	3.6 y	3.7 y	3.4 y	3.5 y	0.3	< 0.001
16:0	29.0 y	31.3 z	31.1 z	28.5 y	32.1 z	30.6 zy	30.4 zy	0.6	< 0.001
18:0	5.8 x	6.9 zy	6.6 yx	6.3 yx	7.5 z	7.6 z	7.0 zy	0.2	< 0.001
SFA[a]	42.8 z	43.2 z	42.5 z	39.4 y	44.2 z	42.6 z	41.9 z	0.7	< 0.001
16:1	7.8 z	3.9 y	3.6 y	3.6 y	3.4 y	3.2 y	3.4 y	0.3	< 0.001
18:1n-7	3.0 z	2.4 y	2.0 x	2.0 x	2.2 yx	2.2 yx	2.1 yx	0.1	< 0.001
18:1n-9	14.0 w	26.7 z	23.3 y	20.2 x	23.6 y	20.9 x	19.4 x	0.7	< 0.001
20:1n-9	0.6 v	1.6 z	1.1 xw	1.0 w	1.4 zy	1.2 yx	1.0 w	0.1	< 0.001
MUFA[b]	26.6 w	36.1 z	31.2 yx	28.2 w	32.1 y	29.0 xw	27.1 w	0.7	< 0.001
18:2n-6	7.5 x	9.7 y	10.3 y	15.7 z	10.2 y	10.9 y	10.1 y	0.4	< 0.001
20:4n-6	0.7 y	0.3 x	0.3 x	0.3 x	0.3 x	2.9 z	3.1 z	0.1	< 0.001
n-6[c]	8.7 w	10.4 x	10.9 x	16.4 z	10.8 x	14.4 y	13.8 y	0.5	< 0.001
18:3n-3	1.2 x	0.7 x	7.6 z	6.0 y	0.8 x	0.9 x	0.8 x	0.2	< 0.001
18:4n-3	1.1 z	0.2 y	0.2 y	0.3 y	0.2 y	0.3 y	0.3 y	0.0	< 0.001
20:4n-3	1.1 z	0.2 y	0.3 y	0.3 y	0.3 y	0.3 y	0.3 y	0.0	< 0.001
20:5n-3	3.4 z	0.9 x	0.7 x	0.9 x	0.9 x	0.9 x	2.4 y	0.1	< 0.001
22:6n-3	11.0 z	6.4 y	5.1 y	6.6 y	8.9 z	9.8 z	10.8 z	0.7	< 0.001
n-3[d]	20.4 z	9.3 v	14.6 yx	14.9 yx	12.0 w	13.1 xw	16.5 y	0.8	< 0.001
PUFA[e]	30.6 zy	20.7 v	26.3 v	32.4 z	23.7 wv	28.4 yx	31.0 zy	0.9	< 0.001
C_{18} PUFA[f]	10.2 w	10.8 xw	18.3 y	22.1 z	11.3 xw	12.1 x	11.3 xw	0.5	< 0.001
LC-PUFA[g]	19.2 z	8.9 xw	7.2 w	9.2 xw	11.4 x	15.4 y	19.0 z	0.9	< 0.001
n-3 : n-6	2.3 z	0.9 x	1.3 y	0.9 x	1.1 yx	0.9 x	1.2 y	0.1	< 0.001
20:3n-9 : 20:4n-6	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]	ND[i]
22:5n-6 : 22:6n-3	0.024 x	0.022 x	0.022 x	0.022 x	0.014 w	0.050 z	0.037 y	0.022	< 0.001

Table 3.9, continued.

[a] Saturated fatty acids—sum of all fatty acids without double bonds; include 17:0, 20:0, 22:0, 23:0 and 24:0 in addition to individually reported SFA

[b] Monounsaturated fatty acids—sum of all fatty acids with a single double bond; include 14:1, 17:1, 22:1n-11 and 22:1n-9 in addition to individually reported MUFA

[c] Sum of all n-6 fatty acids

[d] Sum of all n-3 fatty acids

[e] Polyunsaturated fatty acids—sum of all fatty acids with ≥ 2 double bonds; include 16:2n-4, 16:3n-4, 18:3n-6, 18:3n-4, 20:2, 20:3n-9, 20:3n-6, 22:2, 22:5n-6 and 22:5n-3 in addition to individually reported PUFA

[f] Sum of all PUFA with chain lengths of 18 carbon atoms PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[g] Long-chain PUFA—sum of all fatty acids with chain length ≥ 20 carbon atoms and double bonds ≥ 3

[h] LC-PUFA diet amended with ethyl esters of DHA, ARA, and EPA

[i] ND – Fatty acid not detected

Table 3.10. Coefficient of distance values for tissues of Florida Pompano fed diets containing fish oil, hydrogenated soybean oil, or hydrogenated soybean oil amended with ethyl esters of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (ARA), or eicosapentaenoic acid (EPA)[1]

	FISH OIL (+) CONTROL	EFA-Free (-) Control	ALA	C_{18} PUFA	DHA	ARA + DHA	LC-PUFA
Fillet	0.0	16.8	15.0	14.2	14.2	12.5	10.7
Liver	0.0	26.8	25.3	23.5	14.2	13.9	12.3
Brain	0.0	4.0	3.6	3.5	2.0	3.9	3.2
Eye	0.0	15.1	14.6	13.7	12.5	10.7	8.9
Intraperitoneal fat	0.0	13.9	10.2	12.2	10.4	11.8	10.1

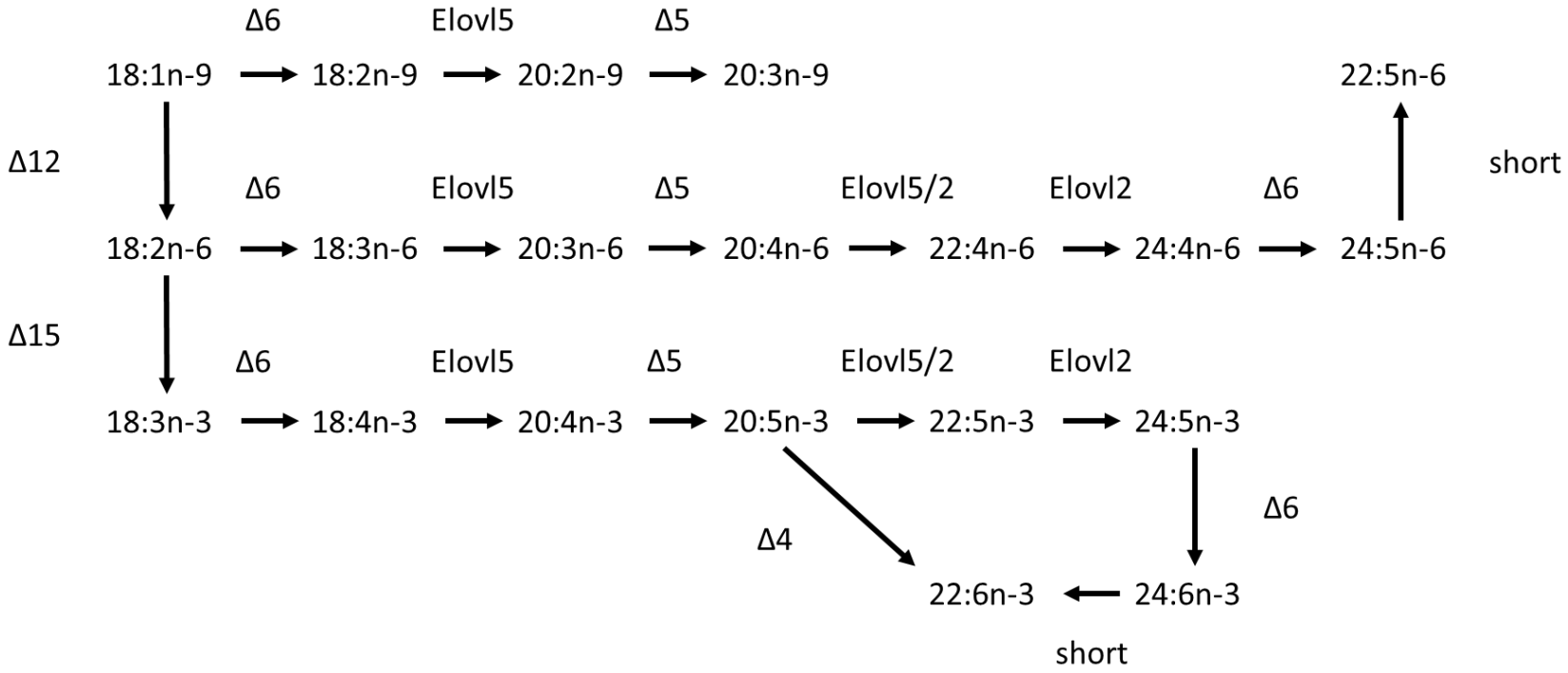

Figure 1.1: Pathways of long chain PUFA synthesis from n-3, n-6, and n-9 C_{18} PUFA; Δ5 and Δ6, fatty acid desaturases; Elovl5 and Elovl2, PUFA elongases; short, beta-oxidized chain shortening (Tocher 2010)

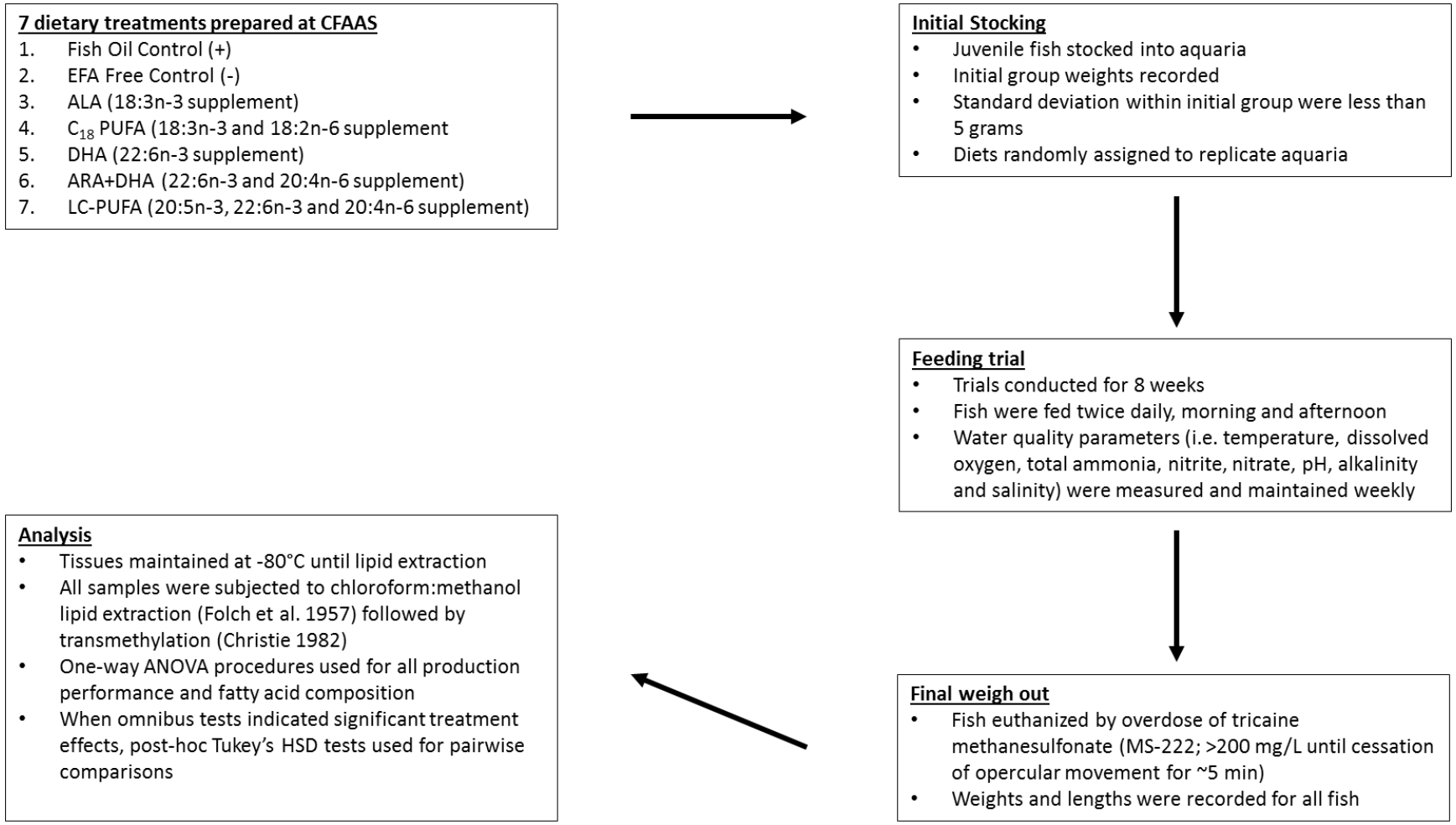

Figure 2.1: Generalized experimental design for Nile Tilapia and Florida Pompano trials

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